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Vol. 8, No. 1, pp. 1-110 July-August, 1961

CONTENTS

	PAGE	RUSS. PAGE
Synthesis of Chlorophyll and Fixation of Carbon Dioxide in Etiolated Barley Seedlings under Illumination. N. G. Doman, A. A. Krasnovskii, A. K. Romanova, L. M. Vorob'eva, E. V. Pakshina, and Z. A. Terent'eva	1	3
Dependence of Photosynthesis and Respiration of Sugar Beets on the Plant Stand Density under Irrigation Conditions. G. A. Evtushenko, L. A. Shpota, and E. S. Shkaf	9	13
On the Formation and Efficiency of the Photosynthetic Apparatus of Agricultural Plants during the Vegetative Period. A. A. Nichiporovich and M. P. Vlasova	13	19
Investigations of Aerobic Metabolism of Sugars and Aerobic Respiration of the Meristem of Roots. K. Ramshorn	19	29
Temperature Dependence of Dormant Seed Respiration. M. G. Nikolaeva and Yu. Yu. Katkevich	28	42
Effect of Water Supply on the Absorbing Activity and Respiration Rate of the Root System of Corn. N. S. Petinov and N. F. Berko	34	51
The Dormant Period and Winter Hardiness of Apple Trees in Western Siberia. A. A. Khristo	39	58
The Effect of Anions on Cotton under Conditions of Gravel Culture. E. F. Ivanitskaya	45	67
Synthetic Activity of the Root System of Corn during Ammonia and Nitrate Nutrition. N. B. Bekmukhamedova	51	75
On the Dormancy of Plants under Conditions of Irrigation with Salt Solutions. B. P. Strogonov and E. Z. Oknina	55	79
The Synthesis of Some Thiourea Derivatives and the Determination of their Activity as Growth Substances. B. T. Markin	61	86
On the Role of Physiologically Active Substances of the Phytohormone and Vitamin Types in Heterosis Phenomena in Corn. F. F. Matskov and S. G. Manzyuk	65	92
Effect of Derivatives of Nucleic Acid Metabolism on the Growth and Flowering of Perilla nankinensis. M. Kh. Chailakhyan, R. G. Butenko, and I.I. Lyubarskaya	71	101
On the Vitality of Sections of Lemon Leaves. G. A. Samygin and N. M. Matveeva	81	114
BRIEF COMMUNICATIONS		
Carbohydrate Metabolism in Wild Chinese Apple Trees during the Period after Planting and its Importance for their Subsequent Growth. N. I. Federov	83	117
Exchange of Substances between Neighboring Higher Plants and Soil Algae. E. K. Uzorin	86	119
Transpiration in Pine Trees of Various Species Showing Natural Hybrid Grafting. M. M. Beskaravainyi	89	122
The Chemical Structure and Herbicidal Activity of Some Derivatives of Phenylhydroxylamine. Yu. A. Baskakov	92	125

CONTENTS (continued)

	PAGE	RUSS. PAGE
METHODS		
Apparatus for Studying Transient Phenomena of Photosynthesis by means of the Carbon Radioisotope C^{14}. V. E. Semenenko	95	129
Method of Studying Rate of Water Exchange (Total Consumption of Water by Soil and Plant) in Field and Laboratory Conditions. D. I. Kolpikov and P. P. Teterin	100	134
Refractometric Method of Estimating Water Content of Leaves of Apple Trees. L. A. Filippov	103	138
CURRENT EVENTS		
The First Siberian Conference of Plant Physiologists and Biochemists	106	141
Recollections of Arkadii Ivanovich Potapov. P. A. Genkel'	109	143

SYNTHESIS OF CHLOROPHYLL AND FIXATION OF CARBON DIOXIDE IN ETIOLATED BARLEY SEEDLINGS UNDER ILLUMINATION

N. G. Doman, A. A. Krasnovskii, A. K. Romanova, L. M. Vorob'eva, E. V. Pakshina, and Z. A. Terent'eva

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The relation between the formation and accumulation of chlorophyll in etiolated plant seedlings and the commencement of photosynthesis long ago attracted the attention of investigators.

The first attempts to determine the liberation of oxygen by etiolated leaves of plants while illuminating them did not lead to definite results, probably due to the imperfection of the methods applied. In studies of the last decade [1-3], it was shown that etiolated leaves of cereals from the very first minutes of illumination acquire a completely measureable ability to liberate oxygen. Simultaneously, the commencement of the accumulation of chlorophyll a is also observed, while chlorophyll b is formed considerably later [3].

The old data obtained in the attempt to compare the commencement of chlorophyll accumulation in greening plant leaves with the commencement of CO_2 fixation are also contradictory. Thus, data are available that photosynthetic assimilation of carbon dioxide by etiolated barley and bean leaves commences only after an appreciable amount of chlorophyll is formed in them [4]. However, Willstätter and Stoll [5] showed that greening etiolated leaves have a greater assimilation number and consequently a greater assimilating ability than the undarkened leaves.

A detailed investigation with the use of labeled carbon dioxide is given in [6], in which the rate of chlorophyll accumulation was compared with the rate of $C^{14}O_2$ fixation during the greening process of etiolated wheat seedlings. In addition to this, the labeled products formed in the leaves after the lapse of different time intervals from the start of illumination were also studied. According to the data of the authors [6], the intensified formation of chlorophyll in the etiolated wheat seedlings commences 2-3 hr after the start of illumination and continues for the next 20 hr at a decreasing rate. The rate of CO_2 fixation remains very low for 1-2 hr after the beginning of the intensified formation of chlorophyll, and 5 hr after the start of illumination only 0.1% of the radioactivity is being assimilated by the completely greened leaves. On further illumination, the rate of $C^{14}O_2$ fixation increases in proportion to the duration of illumination for at least 32 hr from the start of illumination. The authors consider that the plant acquires a slight ability for photosynthetic fixation of $C^{14}O_2$ only after 5 hr of illumination. The authors consider the low content of sugars at this stage as a lack of "reducing agents" or active enzymes for rapid reduction of phosphoglyceric acid to hexoses. In the interval from 1-2 to 5 hr of greening, conditions are developed necessary for the accomplishment of photosynthesis and consist of the accumulation of phosphoglyceric acid and alanine, as a result of which a shift in the equilibrium of the metabolic reactions toward the side of sucrose synthesis occurs.

Investigation of the spectral properties of chlorophyll during its formation and accumulation in etiolated Phaseolus seedlings in light showed that the state of the pigment changes with greening of the seedlings [7,8]. The chlorophyll being formed in the first minutes of greening has a maximum absorption at 670 mμ. With an increase in greening and accumulation of chlorophyll, the maximum gradually shifted to the long-wave side of the spectrum, to 677-678 mμ – the usual position of maximum absorption of chlorophyll in green leaves.

The task of our investigation included a more detailed study of the relation between the rate of $C^{14}O_2$ fixation in light and in dark and the accumulation of chlorophyll, with simultaneous observations of the change in its form which occurs during greening of the etiolated leaves. We also wanted to detect how the composition of the carbon fixation products changes during the transition from a heterotrophic type of metabolism in etiolated leaves to an autotrophic type of photosynthetic metabolism.

METHODS

Eight-day-old etiolated barley seedlings were illuminated with an incandescent lamp (150 w) located at

a distance of 1.3 m. The samples for determining the chlorophyll content were selected at certain time intervals after the start of greening. Etiolated seedlings were also investigated. At the same time experiments were set up for the fixation of $C^{14}O_2$ in light and in the dark. Samples of barley leaves grown in light were taken as an additional control.

The chlorophyll content was determined by the spectrophotometric method [7].

The state of the chlorophyll at a given stage of greening was judged by the position of the long-wave absorption maximum of chlorophyll in a homogenate—colloidal solution of the substance of etiolated and greening leaves. The colloidal solution was prepared in the following manner: 0.5 g of leaves was pulverized with 6 ml of 0.067 M Na_2HPO_4 (pH 8.5). The pasty mass was squeezed through linen and centrifuged five minutes at 3500 G. All the described operations were carried out in a darkened room. The absorption spectrum of the colloidal solution obtained was measured on a Beckman spectrophotometer. The accuracy of measuring the position of the maximum was ± 1 mμ.

The rate of $C^{14}O_2$ fixation by greening barley leaves in light and in the dark was investigated in a chamber floating over mercury [9]. The volume of the gas space of the chamber was 200 cm^3; the carbon dioxide content of the gas in the chamber was 0.5%.

While studying the rate of photosynthesis the chamber was illuminated by a 500 w incandescent lamp located 70 cm from the chamber. In order to exclude the possibility of the effect of yellow pigments, the light flux was passed through a KS-11 red light filter.

When determining the intensity of $C^{14}O_2$ fixation in the dark the greening leaves were preliminarily exposed in darkness for ten minutes. The surrounding temperature while conducting the experiment was 24-26°.

Exposure of the leaves in an atmosphere containing $C^{14}O_2$ was five minutes. Six leaves with a total weight of 200 mg were selected for each exposure.

Fixation of the leaves was accomplished by immersion in boiling acidified ethanol (10 ml 0.1 N HCl per 90 ml 96% alcohol). The alcohol-soluble substances were extracted by a sixfold treatment with hot acidified alcohol. The combined alcoholic extracts were evaporated until dry in a vacuum drier over dry alkali. The dry residues were dissolved in a determined volume of acidified alcohol, and the radioactivity was determined in part of the solution by means of a Geiger-Mueller end-window counter.

Separation of the experimental solutions into fractions was carried out with the use of KU-1 cation-exchange resin under static conditions. The substance absorbed by the resin was eluted with 6 N ammonia solution.

The products of $C^{14}O_2$ fixation were investigated by the method of paper chromatography in conjunction with autoradiography.

RESULTS

The data obtained upon determining the rate of chlorophyll accumulation, the position of the long-wave maximum absorption, and also the radioactivity of the experimental samples during $C^{14}O_2$ fixation in light and in the dark are shown in Fig. 1. The accumulation of chlorophyll in greening barley leaves occurs uniformly during the first two days from the start of illumination, increasing during this period from 0.03 to 1.3 mg converted per 1 g of dry weight, while green, undarkened barley leaves of the same age contain 1.7 mg of chlorophyll per 1 g of dry leaves.

The maximum absorption of chlorophyll, which at the start of greening was at 670 mμ, after a lapse of 2 hr of greening shifted to 672 mμ and in 20 hr reached 675 mμ. For green leaves the maximum is located at 676 mμ. The initial changes in the maximum absorption of chlorophyll under the given method of the experiment were not observed; they occurred in the time interval before the formation of the form with the maximum at 672 mμ. Thus the over-all picture of the accumulation of chlorophyll and the shift of the long-wave maximum of its absorption proved to be very similar for barley and Phaseolus [8,9].

Figure 1 also shows the total radioactivity of the samples with $C^{14}O_2$ fixation in the dark and photosynthesis in red light. During the first hour of illuminating the etiolated barley leaves, the fixation of tagged carbon dioxide in the light and in the dark were represented by values of the same order from 1.2×10^5 to 2.3×10^5 disintegrations per minute fixed per 1 g of dry leaves.

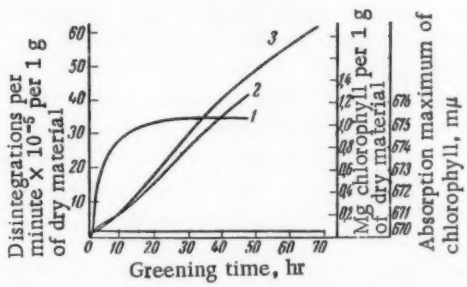
Disintegrations per minute × 10⁻⁵ per 1 g of dry material
Mg chlorophyll per 1 g of dry material
Absorption maximum of chlorophyll, mμ
Greening time, hr
1
2
3

Fig. 1. Rate of chlorophyll accumulation, the change of the position of the long wave maximum of its absorption, and the rate of $C^{14}O_2$ fixation in light and in the dark during the greening process of etiolated barley leaves. 1) Change in the position of the absorption maximum of chlorophyll; 2) chlorophyll content; 3) rate of $C^{14}O_2$ fixation in light: line parallel to the axis of the abscissa is the rate of $C^{14}O_2$ fixation in the dark.

TABLE 1. Distribution of Radioactivity by Fractions with Treatment of the Aqueous Extracts of Greening Etiolated Barley Leaves with KU-1 Cation Exchanger (in % of the radioactivity of the water-soluble fraction)

Barley leaves after greening	Conditions of $C^{14}O_2$ fixation	Fraction of substances not adsorbed by the KU-1 resin	Ammonia eluate from resin	Losses of radioactivity on treatment with the resin
30 min	In the dark	35.0	53.0	12.0
	In the light	41.8	37.9	20.3
69 hr	In the dark	55.6	30.1	14.3
	In the light	66.6	11.0	22.4

Two hours after the start of illumination an increase was noted in the rate of $C^{14}O_2$ fixation in light, continuing in proportion to the duration of greening at least for the next 60-70 hr. After 69 hr of greening the rate of photosynthesis of leaves reaches the level of $C^{14}O_2$ fixation by barley seedlings grown in light, equal to $7 \times \times 10^6$ disintegrations per minute per 1 g of dry leaves during a five-minute exposure to labeled carbon dioxide.

The rate of $C^{14}O_2$ fixation in the dark by etiolated barley leaves does not substantially change during their greening process and, as a rule, does not exceed 2% of the rate of photosynthesis of completely green leaves.

It is necessary to note that several series of similar experiments were set up with eight-day-old etiolated seedlings of Phaseolus. In these experiments the same regularities of accumulation and changes in the state of chlorophyll were observed as in all previous experiments with Phaseolus and barley. In regard to $C^{14}O_2$ fixation, a comparatively slight increase in light during the greening process of the leaves proved to be characteristic. Therefore for Phaseolus seedlings we can note only a small excess in photosynthetic fixation of $C^{14}O_2$ in comparison with fixation in the dark, the excess having, after 6 hr of greening, a value of the order of 7%, and after 10 hr of greening, 24%.

It is possible to assume that the detected difference in the increase in the photosynthetic rate in barley and Phaseolus is related to the retarded development of the photosynthetic apparatus and with the general disruption of metabolism in the etiolated Phaseolus, which is reflected outwardly by the sharp inhibition of the development of this plant. In future experiments, therefore, it was necessary to reject such an object.

Table 1 shows comparative data, obtained while treating the experimental solutions with the KU-1 cation exchanger, reflecting the distribution of radioactivity by fractions in barley leaves which scarcely started to turn green (after 30 min of illumination) and in completely green barley leaves (after 69 hr of illumination) with fixation of the tagged carbon dioxide for 5 min in the dark and in the light.

It is apparent from the data in Table 1 that even after 30 min of greening a definite difference is noted in the quantitative distribution of radioactivity in etiolated barley leaves between the labeled carbon dioxide fixation products in the light and in the dark. This difference increases with an increase in the greening of the leaves.

With $C^{14}O_2$ fixation in the dark by etiolated barley leaves, after 30 min of their greening more than half of the tagged products (53%) were detected in the ammonia eluate from the KU-1 cationite, mainly consisting of amino acids.

With $C^{14}O_2$ fixation by these same leaves in light, in the ammonia eluate a smaller but still considerable portion of the total radioactivity of the aqueous extract (about 38%) was detected.

With $C^{14}O_2$ fixation by green leaves in the dark, this fraction was about 30% of the total radioactivity of the extract. The lowest per cent (11%) of the total radioactivity was the fraction of amino acids synthesized during photosynthesis of green leaves.

Generally within a single period of greening in the dark with the given exposure to $C^{14}O_2$ the substances of the fraction adsorbed by the KU-1 cationite were tagged relatively more intensely than in light.

However, the percent of radioactivity detectable in the solution after removal of the amino acids and different bases by the resin is greatest in photosynthesizing green leaves, as shown in Table 1: with photosynthesis of green leaves 66.6%; with fixation of $C^{14}O_2$ in the dark by green leaves 55.6%; and in etiolated leaves after 30 min greening with $C^{14}O_2$ fixation in light 42% and with fixation in the dark, 35%.

TABLE 2. Distribution of the Radioactivity among Products Contained in the Ammonia Eluate from the KU-1 Cation Exchange Resin Relative to the Greening Period of Barley Leaves (in % of the radioactivity of the ammonia eluate)

Barley leaves after greening	Conditions of $C^{14}O_2$ fixation	Unknown substances at the origin	Zone of aspartic acid	Zone of serine	Zone of glutamic acid	Zone of glycine and asparagine	Zone of α-alanine	Group of products rapidly moving in phenol
30 min	In the dark	6.7	22.4	11.5	20.5	4.4	2.2	32.1
	In the light	7.9	23.5	13.1	19.7	3.8	2.4	29.5
69 hr	In the dark	13.5	15.2	6.9	8.0	1.5	4.6	50.3
	In the light	28.6	6.8	24.7		8.6	6.3	28.0

It is necessary to note that when treating the radioactive solutions with KU-1 resin recovery of the radioactivity was incomplete. The losses can be explained, on one hand, by the irreversible adsorption of certain substances on the ion exchanger and, on the other hand, by the partial decomposition of products not stable with respect to strong acids with liberation of C^{14} as gaseous products, since it is known that strongly acidic sulfonated carbon compounds to which the KU-1 type ion exchanger is related have properties of strong sulfuric acid [10].

It is apparent from the data in Table 1 that the relative loss of radioactivity occurring during treatment of the radioactive solutions with the exchanger was considerably less during $C^{14}O_2$ fixation in the dark than the corresponding loss during photosynthesis of greening barley seedlings.

In order to find possible differences in the qualitative or quantitative ratio of the individual radioactive substances, we studied the chromatograms of amino acids eluted from the resin. The solvent for chromatography was phenol-0.1 M formic acid which was passed over the paper three times for better separation of the substances. Spots corresponding to the position of the markers of the amino acids were cut out from the chromatograms and their radioactivity determined in a 4-π type gas-flow counter.

It is apparent from the data in Table 2 that the relative distribution of radioactivity in the saturated ammonia eluate of barley leaves after 30 min greening is approximately identical for $C^{14}O_2$ fixation in the dark and in light, while in green barley leaves with fixation in the dark there is a noticeable decrease in the relative content of labeled substances in the zone of aspartic and glutamic acids; the marker somewhat increases in the zone of alanine and is close to the origin (possibly peptides).

During $C^{14}O_2$ fixation by green leaves in light, these changes were expressed still more clearly: the content of tagged substances at the origin reached 28.6% of the total radioactivity of the eluate from the ion exchanger,

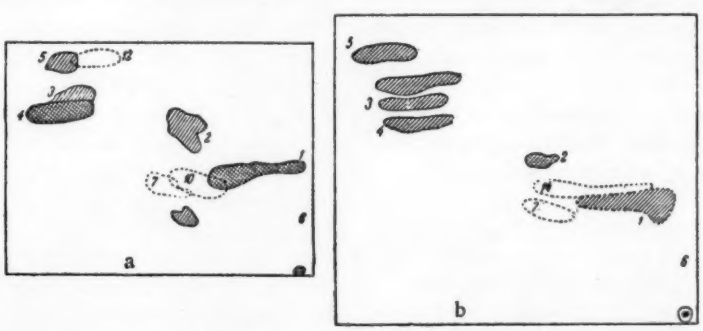

Fig. 2. Products of $C^{14}O_2$ fixation in the dark by greening barley leaves. Duration of greening: a) 1 hour; b) 2 hours. 1) Aspartic acid; 2) malic acid; 3, 4) unknown amino acids; 5) succininc acid; 6) zone of phosphate esters of sugars and phosphoglyceric acid; 7) sucrose; 8) α-alanine; 9) valine; 10) serine; 11) citric acid; 12) α-ketoglutaric acid; 13) fumaric acid; 14) glutamic acid; 15) γ-aminobutyric acid; 16) glucose; 17) fructose; 18) β-alanine; 19) phenylalanine; 20) proline. Exposure in $C^{14}O_2$ was 5 min.

the percent of radioactivity in the region of aspartic acid dropped to 7, while the relative content of the tagged α-alanine reached more than 6%.

It is necessary to point out that the composition of the group of tagged products rapidly moving in phenol saturated by 0.1 M formic acid is varied with respect to the conditions of $C^{14}O_2$ fixation.

When the autoradiographs from the chromatograms of the aqueous extracts of the etiolated barley leaves which fixed the $C^{14}O_2$ in the dark were obtained, the x-ray film revealed prints of six or seven of the most intensely labeled substances. Fixation of labeled carbon dioxide in the dark was characterized by considerable radioactivity of the aspartic acid as compared with other radioactive products in the extract. It was also possible to detect clearly the radioactivity in malic and succinic acids (Fig. 2). A further, more precise identification of the labeled amino acids by adsorption of the fraction of substances containing the basic groupings by means of the KU-1 resin with their subsequent elution with ammonia made it possible for us to detect up to ten products of $C^{14}O_2$ fixation in the dark. These included aspartic acid, two substances unidentified by us, and glutamic acid. It was characteristic that α-analine was relatively weakly labeled during $C^{14}O_2$ fixation in the dark.

Although during the greening process of barley leaves the qualitative composition of the products of $C^{14}O_2$ fixation in the dark did not vary greatly, a definite change did occur in their quantitative relations. First of all the relative content of radioactivity in the zone of phosphate esters increased and the percentage of amino acids decreased (see Table 1 and Figs. 2 and 3).

Also characteristic was the relative decrease in radioactivity in the zones of the aspartic and glutamic acids and an increase in the zones of alanine and peptides. However, the amount of aspartic acid from carbon dioxide fixation in the dark remained considerable in all cases. Even in the very early stages of the greening of barley seedlings it was possible to detect very weak, but detectable, radioactivity on the chromatograms in the zone of phosphate esters of sugars.

The possibility of the occurrence of radioactive phosphate esters during fixation of labeled carbon dioxide in the dark, especially in extracts from green leaves, can hardly be disputed. In the literature, cases are noted of the occurrence of some radioactivity during $C^{14}O_2$ fixation in the dark not only in phosphate esters of sugars, but also in free sugars [11, 12]. The occurrence of radioactivity in phosphate esters of carbohydrates can be explained by the presence of the remains of a light type of metabolism whereby the longer the greening period and, consequently, the more intensive the light inclusion of carbon dioxide, the richer the cells are in substrates and "reducing agents" and the longer the traces of an autotrophic type of metabolism are retained.

On the other hand, in vigorously growing seedlings radioactivity in phosphate esters can occur as the result of the reversibility of the glycolysis reactions, although the trend of processes characteristic for plants toward intensive consumption of carbohydrates in the dark makes this means less probable. During $C^{14}O_2$ fixation in the dark under conditions of our experiments, we did not detect radioactivity in sucrose, glucose, fructose, valine, proline, histidine, serine, glycine, tyrosine, leucine, γ-hydroxybutyric acid, isoleucine, in citric fumaric, aconitic, α-ketoglutaric, or glycolic acids, or in the free organic bases adenine, guanine, uracil, thymine, or cytosine.

An examination of the series of radiochromatograms of the extracts from the greening barley leaves permits us to make certain conclusions on the order of occurrence of labeled products characteristic for the autotrophic type of carbon assimilation.

The character of the "photosynthetic" products of the etiolated barley leaves (Fig. 4a) did not differ from the composition of labeled substances formed by $C^{14}O_2$ fixation in the dark. During the first days of greening

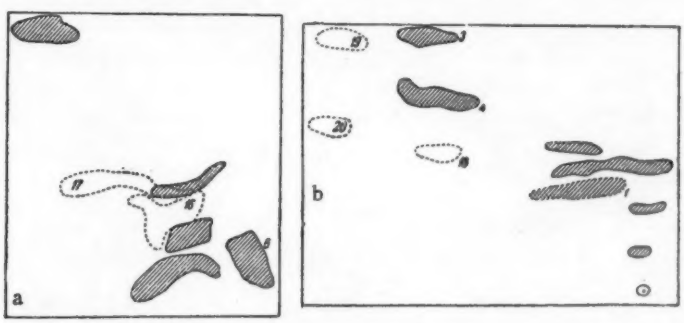

Fig. 3. Distribution by fractions of the labeled products of $C^{14}O_2$ fixation in the dark by greening barley leaves. Duration of greening was 6-8 hours. a) Neutral and acidic products not adsorbed by the KU-1 resin; b) basic products eluted from the resin with 6 N ammonia. Remaining legend as in Fig. 2.

there was an appreciable increase in the ability of barley leaves to form phosphate esters, with a simultaneous decrease in the content of aspartic and malic acids (Fig. 4b). After illuminating the leaves for 2 hr (Fig. 4c) a labeled substance occurred which is very characteristic for carbon dioxide fixation in light by barley, located in the sucrose zone, but which is still unidentified by us. It is possible that the radioactive spots correspond to several products. After the leaves have greened 6 hr (Fig. 4d) the activity in serine, α-alanine and valine became more noticeable. The labeled sugars – glucose and fructose – occurred in noticeable amounts only after the lapse of 69 hr from the start of illumination of the etiolated barley leaves (Fig. 4f).

In completely greened barley leaves we could detect, in addition to glucose and fructose, numerous labeled compounds – phosphate esters of sugars (glucose, fructose, ribose, and, possibly, sucrose), phosphoglyceric and aspartic acids, serine, α-alanine, valine, malic acid – in ratios characteristic for photosynthesizing leaves; i.e., the predominating portion of the radioactivity is contained in the phosphate esters and sugars, while only a small part of the total radioactivity of the extract goes to the amino acids (see Table 1 and Fig. 4f and Fig. 5).

DISCUSSION OF THE RESULTS

The change in composition of the products of carbon dioxide assimilation depicts a replacement of the heterotrophic type of nourishment by an autotrophic type in greening etiolated plant seedlings. From this point of view the occurrence of sucrose or another sugar in a number of products of $C^{14}O_2$ fixation could be the criterion of the commencement of photosynthetic activity. However, it is necessary to refer to experimental results of this nature with great caution, since in this case a decisive role can be played by the duration of exposure to

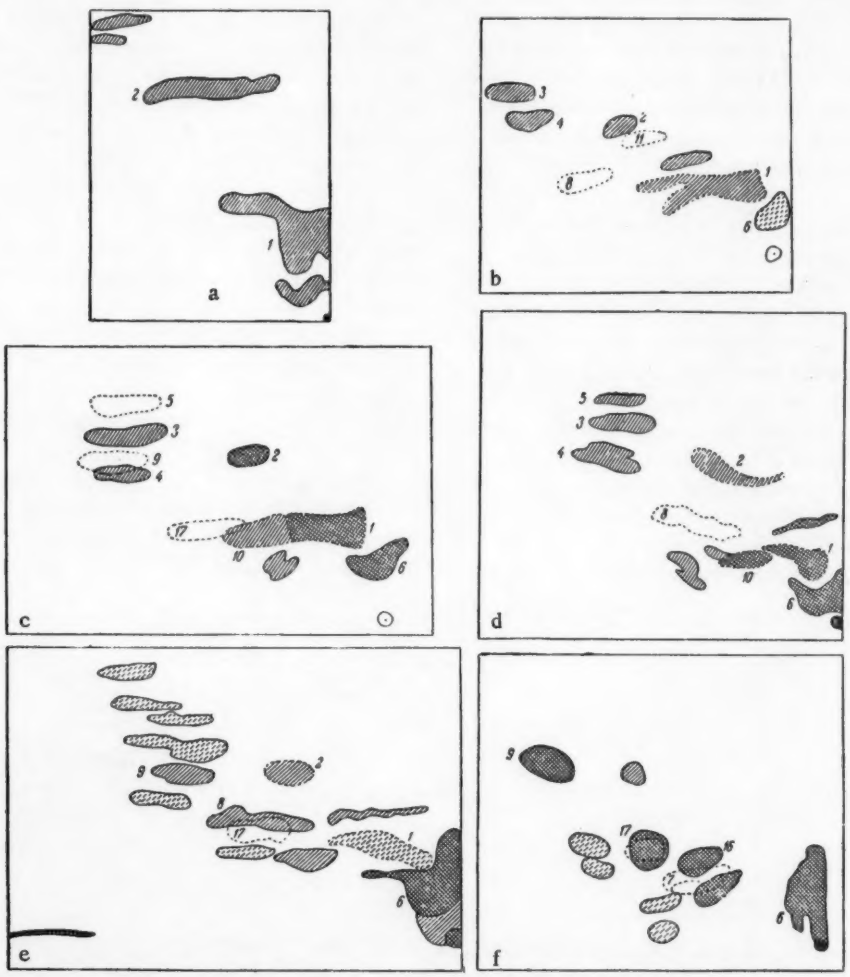

Fig. 4. Change in the composition of the photosynthetic products during the greening process of etiolated barley leaves. a) Etiolated leaves; b) same after 1 hr of greening; c) after 2 hr; d) after 6 hr; e) after 25 hr; f) after 69 hr. Remaining legend as in Fig. 2.

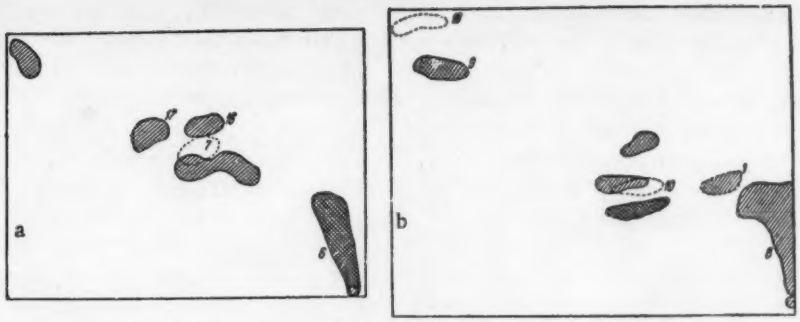

Fig. 5. Distribution by fractions of the labeled products of photosynthesis by barley leaves after 69 hr of greening. a) Neutral and acid products adsorbed by the resin KU-1; b) basic products eluted from the resin with 6N ammonia. Remaining legend as in Fig. 2.

carbon-labeled carbon dioxide, which is selected arbitrarily by the investigator. With a general retardation of the metabolic processes in greening leaves, which is expressed, in particular, by the low rate of photosynthesis, the delay in the occurrence of the marker in sugars can be erroneously attributed to the inability of leaf tissue to photosynthesize.

In order to obtain comparable results when working with different objects an accurate standardization of the conditions of setting up the experiments is necessary (the intensity of illumination during the greening process and at the instant of labeled carbon dioxide fixation, the surrounding temperature, the concentration of $C^{14}O_2$ and the duration of exposure to it, etc.).

In all probability, the elementary ability for autotrophic assimilation of carbon dioxide is inherent in seedlings just beginning to turn green. The latest data in the literature on the commencement of oxygen liberation and chlorophyll accumulation immediately after the start of illumination of etiolated leaves [2, 3] and on the early finding of traces of radioactivity in phosphate esters after feeding of the leaves with ribose [6], as well as our data on the differences in the distribution of the marker by fractions after 30 min of illumination testify to the fact that a partial switch from a dark regime of carbon assimilation to a light regime already occurs in the presence of a negligible amount of chlorophyll. Consequently, it is necessary to propose the question not on the start of specifically photosynthetic activity, but on the quantitative relation of the autotrophic (photosynthetic) and heterotrophic (dark) types of metabolism and on the determining role of both of them.

SUMMARY

1. The accumulation of chlorophyll starts from the very first minutes of illumination of etiolated barley leaves and continues at an optimum regime of illumination for two days from the start of illumination; the maximum of the absorption spectrum of chlorophyll in homogenates during the greening process of barley leaves gradually shifts from 670 to 676 mμ.

2. An appreciable difference between the amount of $C^{14}O_2$ fixation in light and in the dark occurs after illuminating the etiolated barley leaves for 2 hr, i.e., with the formation of the form of chlorophyll with maximum absorption at 670-672 mμ.

The rate of $C^{14}O_2$ fixation in the dark is practically independent of the degree of greening of barley leaves. Fixation of $C^{14}O_2$ in light increases as the barley leaves become green and after 70 hr reaches the photosynthetic rate of green leaves.

3. As the leaves become green, changes occur in the quantitative relation of the $C^{14}O_2$ fixation products in the dark, although their qualitative composition is essentially unchanged.

4. Differences in the relative amount of $C^{14}O_2$ fixation products in light and in the dark can already be noted after 30 min of greening of the barley leaves. Transition from a heterotrophic type of metabolism to the dominant autotrophic type in barley leaves during their greening occurs gradually over 2-3 days.

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DEPENDENCE OF PHOTOSYNTHESIS AND RESPIRATION OF SUGAR BEETS ON THE PLANT STAND DENSITY UNDER IRRIGATION CONDITIONS

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Photosynthesis and respiration—these are the most important physiological processes, the level and relationship of which to a great degree determine the growth, chemical composition, and over-all plant yield. The study of photosynthesis and respiration of sugar beets under specific irrigated cultivation conditions is therefore not only of considerable theoretical interest, but also of practical interest.

The results of such a study permit us to determine the nature of the dependence of these processes on the soil-climatic and agricultural engineering conditions of our sugar beet growing, and to develop scientific bases for increasing the sugar content and yield of sugar beets.

In connection with this, in 1957 we studied photosynthesis and respiration of two varieties of sugar beets—the Yanash sugary nonlocal variety and the less sugary, higher yielding local variety K-08.

The study was conducted under diurnal and seasonal variations directly under field conditions on experimental plots of the Kirgiz beet experimental station against a background of optimum irrigation with two plant stand densities: a) 45 × 18 cm, according to the agricultural rules a normal stand density; and b) 45.5 × 45.5 cm, a thinned stand density.

Photosynthesis was studied by means of specially designed field chambers with the use of C^{14}. For these purposes we took circular cuttings with a diameter of 2 cm from blades of the middle stratum of leaves by means of a metal form. The cut-outs were immediately placed in a closed chamber and $C^{14}O_2$ introduced. We held them there in the light for 5 min, then fixed them in hot alcohol vapors, transferred them to special packages and sent them to the laboratory where carefully, with the necessary precautions, we pulverized them.

We determined the isotopic activity in them on T--25-Bf1 end-window counters of the B-2 installation.

We calculated the rate of photosynthesis in counts per minute per 60 mg of dry substance. The respiration rate was determined in the same leaves also directly in the field and simultaneously with calculation of photosynthesis. Calculation of respiration was carried out using barium hydroxide absorption of CO_2. The results were expressed in mg of CO_2 per 1 g dry substance per hour.

The samples were taken on Aug. 19, 23, 30 and Sept. 12 at the following hours: 10:30 AM and 1:30, 4:30, and 6:30 PM. On the last date, at 6:30 PM, photosynthesis and respiration were not calculated because it rained.

We determined the chlorophyll content only on August 30 and September 12. The determination was carried out in an acetone extract on an SF-4 spectrophotometer. The amount of chlorophyll was expressed in mg per dm^2 of leaf area. The meteorological indices and illumination were taken into consideration each time (Table 1).

The results of the calculation of photosynthesis and respiration under diurnal and seasonal variations are given in Tables 2 and 3.

It is apparent from Table 2 that regardless of the density of the stand for the Yanash variety the plants are distinguished by the highest photosynthesis in the morning hours and the lowest in the midday or evening hours. The fact of the sharp decrease during midday hours indicates the presence in this variety, under our conditions, of a midday photosynthetic depression. This midday depression was especially exhibited on August 23, when the air temperature was highest. Data are available in the literature which indicate that under the conditions of Uzbek SSR during the hot summer growing periods, when the temperature is above 40°, photosynthesis in sugar beets declines until it completely stops [1]. Our attention is attracted to the fact that in this variety the daytime maximum of photosynthesis occurs only in the morning hours, when the meteorological factors are less intense. Apparently the midday and afternoon conditions of a hot day are very unfavorable for photosynthesis in this variety. At the same time, during the midday and evening hours the plants of the Yanash variety are distinguished by a higher level of respiration. It follows from this that photosynthesis not only sharply declines in the indicated variety during the midday and afternoon depression, but the respiration rate just as sharply increases and, consequently, the efficiency of photosynthesis decreases all the more.

TABLE 1. Meteorological Characteristics

Date of calculation	Air temperature in °C at hr of calculation				Relative air humidity, %				Illumination, lux			
	10 hr. 30 min	13 hr. 30 min	16 hr. 30 min	19 hr	10 hr. 30 min	13 hr. 30 min	16 hr. 30 min	19 hr.	10 hr. 30 min	13 hr. 30 min	16 hr. 30 min	19 hr.
Aug. 19	24.6	27.0	25.0	26.2	46	43	49	46	12160	11120	11120	10480
Aug. 23	24.8	29.6	28.0	22.0	50	37	26	54	11920	12160	10960	2400
Aug. 30	21.8	24.4	24.2	18.0	35	28	27	64	12400	12800	10080	8400
Sept. 12	25.6	27.0	21.0	—	25	45	54	99	11600	10400	7680	rain

TABLE 2. Daily and Seasonal Variations in the Rate of Photosynthesis and Respiration in Sugar Beets Relative to the Density of the Stand

Date of calculation	Photosynthesis				Respiration			
	10 hr 30 min	13 hr 30 min	16 hr 30 min	19 hr	10 hr 30 min	13 hr 30 min	16 hr 30 min	19 hr
Yanash variety – normal density								
Aug. 19	968	130	355	343	—	—	6.56	4.39
Aug. 23	669	99	145	26	6.64	20.9	5.40	19.86
Aug. 30	121	101	101	20	2.69	3.22	4.89	2.36
Sept. 12	269	148	144	—	2.56	6.50	1.67	—
Yanash variety – thinned								
Aug. 19	1010	156	293	412	1.99	6.16	7.32	7.96
Aug. 23	289	150	87	18	2.97	8.42	3.80	13.38
Aug. 30	164	112	479	50	3.35	1.86	6.53	1.36
Sept. 12	479	222	264	—	3.35	2.21	1.39	—
K-08 variety – normal density								
Aug. 19	559	120	657	639	8.76	2.96	5.02	11.45
Aug. 23	792	544	399	45	6.57	7.90	8.85	10.75
Aug. 30	368	377	134	21	1.93	4.57	1.07	0.74
Sept. 12	654	457	81	—	2.40	3.94	1.98	—
K-08 variety – thinned								
Aug. 19	—	575	519	294	3.32	8.52	6.72	2.56
Aug. 23	644	1010	1168	64	7.34	8.19	11.38	10.56
Aug. 30	355	284	673	168	5.09	5.40	3.68	0.86
Sept. 12	814	645	30	—	3.86	7.34	2.89	—

Note: Determination of photosynthesis and respiration was carried out for the first date of calculation (August 12) on the 7th day after irrigation, and for the subsequent dates on the 11th, 19th and 6th day after irrigation, respectively.

Examining the data on the seasonal variation of growing plants, it is easy to note that the lowest photosynthesis is exhibited in the plants of August 30, when the calculation of this index was made on the 19th day after irrigation. On the remaining dates photosynthesis was calculated 6-11 days after irrigation. It is evident that delay in irrigation quite noticeably reduced photosynthesis in this variety—it apparently needs more frequent watering.

It was noted earlier that an appreciable decrease in soil moisture leads to a noticeable decrease in the rate of photosynthesis [2]. In the case of potassium fertilizers, photosynthesis declines to a lesser degree than with nitrogenous fertilizers [2,3].

It is necessary to note that plants that have been thinned are distinguished by higher photosynthesis and by lower respiration. In this case the rate of respiration in normal and thinned stands increases until midday.

The data of Table 2 show that the K-08 variety is distinguished from the Yanash variety by higher photosynthesis, particularly in a thinned stand, and also by a considerably lower midday photosynthetic depression, or by the absence of the latter. This variety is characterized by a lower respiratory rate in a normal stand and higher rate in a thinned stand.

In contrast to the Yanash variety, the daytime maximum of photosynthetic activity in the K-08 variety occurs in both the morning and midday experiment and even the afternoon experiment. It is evident that this variety is more heat resistant than the Yanash variety.

Simultaneously it is necessary to note that for the K-08 variety the plants in the experiment of August 30 are distinguished by the lowest photosynthesis.

Thus, a considerable increase in the periods between watering causes a decline in photosynthesis in both varieties. However, the K-08 variety under such conditions

TABLE 3. Average Indices of Diurnal Variation of Photosynthesis and Respiration for Three Dates of the First Three Daytime Experiments

Variety	Density	Photosynthesis				Respiration				Chlorophyll	Water content, %
		10 hr. 30 min	11 hr. 30 min	16 hr. 30 min	average	10 hr. 30 min	11 hr. 30 min	16 hr. 30 min	average		
Yanash	Normal	353	116	130	199	3.83	9.37	3.99	5.74	5.85	81.5
	Spaced	277	161	277	238	3.22	4.16	3.88	3.75	6.95	81.7
K-08	Normal	604	459	204	423	3.63	6.32	3.97	4.63	5.95	84.8
	Spaced	604	646	623	694	5.42	5.61	5.97	5.87	6.15	84.2

TABLE 4. Average Indices of the Seasonal Variations in the Rate of Photosynthesis and Respiration (for each date) from the First Three Daytime Experiments with Different Plant Stands

Date of calculation	Yanash				K-08			
	photosynthesis		respiration		photosynthesis		respiration	
	normal stand	spaced	normal stand	spaced	normal stand	spaced	normal stand	spaced
Aug. 23	304	175	10.9	5.06	578	941	7.71	7.97
Aug. 30	108	218	3.60	3.91	293	437	2.52	4.99
Sept. 12	187	322	2.71	2.28	397	496	3.63	4.66

has a smaller decline in photosynthesis than the Yanash variety. In addition to this, the K-08 variety is distinguished by higher photosynthesis and by only a negligible midday depression. These facts, as well as the greater heat resistance, apparently explain the higher yield in the K-08 variety than in the Yanash variety under our hot summer conditions.

Marinchik [4] also notes that the sugary Yanash variety under conditions in the Ukraine is distinguished from the R-06 variety by a lower rate of photosynthesis and higher water deficit.

Investigators have observed the midday depression of photosynthesis in sugar beets [4-6] and in other plants [7-9, et al.].

This physiological phenomenon is a result of the fact that during the especially high intensity of the meteorological factors during the midday hours, the transpiration rate increases so much that the plants do not have time to replenish the water deficit developing in the leaves. As a result translocation is delayed, the leaves are overflowing with assimilants, their temperature and respiration considerably increase, and the rate of photosynthesis sharply declines. The plants are less productive the more profound, continuous, and frequent the midday depression of photosynthesis.

Under conditions of the beet growing regions of Kirgizia, on certain clear, sunny summer days the intensity of illumination is so high that the air temperature reaches 38° and higher, which is why the midday retarding of photosynthesis of sugar beets, along with the long midday period, is not a rare event here, and must be taken into account. During hot summer periods the efficiency of photosynthesis is usually higher here under conditions of cloudy weather.

In order to eliminate the overheating of sugar beet leaves during hot summer periods, a fairly successful attempt to whiten them with lime was made [10-12]. However this method still has not found practical application. Apparently for such regions as Kirgizia more frequent ordinary waterings and freshening sprinklings, especially during the hot summer growing periods, and the development of more heat resistant and, consequently, less retardable varieties will be the most effective means to increase the efficiency of photosynthesis of sugar beets during hot summer periods.

In Kirgizia, in addition to the intensity of the illumination causing a considerable increase in temperature and a profound midday retarding as well as overheating of the leaves, the possible afternoon deficit in the CO_2 content in the surface layer of air apparently contributes to the photosynthetic depression in sugar beet crops, especially in still weather.

Such a phenomenon is observed on the sugar beet plantations under conditions of the Ukraine [13].

Table 3 shows the data on the average indices for three daytime periods for the next three experiments (August 23, 30 and September 12), when a calculation of the photosynthesis and respiration was made under diurnal variations.

It is apparent from Table 3 that the K-08 variety, regardless of the plants' density, is distinguished by a higher photosynthesis. Under conditions of a thinned stand

its efficiency of photosynthesis increases to a greater degree than in the Yanash variety. With a normal stand density the Yanash variety is distinguished by a higher rate of respiration, and, with a thinned stand, the K-08 variety.

In both varieties, with a thinned stand the diurnal variation in respiration is more uniform, while with a normal density a sharp increase in this index is observed until midday, then decreases.

In connection with the rate of respiration and photosynthesis relative to the plant density, in both varieties such a dependence is observed under diurnal variations: for the Yanash variety the plants of the thinned stand are distinguished by a somewhat higher photosynthesis and noticeably lower level of respiration, while in the K-08 variety with a considerably higher photosynthesis the thinned plants are distinguished by a higher respiration rate.

Table 4 shows the data on the average indices from the daytime experiments for the next three dates.

It is apparent from Table 4 that the rate of respiration in beets decreases as they grow. In the Yanash variety this index is lower than in K-08 with a thinned stand, and with a normal stand is generally higher.

SUMMARY

1. The sugary Yanash variety differs from the K-08 variety by a lower water content in the leaves, a lower photosynthetic activity, and also by the fact that its daily maximum of photosynthesis occurs in the morning hours, which indicates its weak heat resistance.

2. The respiratory rate in the Yanash variety with a normal stand is generally higher and with a thinned stand lower than in the K-08 variety.

3. In both varieties the rate of photosynthesis is higher under conditions of a thinned stand. Photosynthesis increases most sharply in a thinned stand of the K-08 variety.

4. The sugar beets under conditions of irrigation exhibit a midday depression in photosynthesis, it being less in the K-08 variety and considerably more in the Yanash variety. Here, the latter has a sharp increase in the resparation rate.

5. Long periods between watering lower photosynthesis in the Yanash variety considerably more than in the K-08 variety.

6. The greater yield of the K-08 variety as compared with the Yanash variety is due to the higher photosynthesis, its less acute midday retardation and, apparently, its higher heat resistance.

7. It is necessary to develop and regionalize under conditions of the Chuysk valley more heat resistant, thus less retardable (with respect to photosynthesis), varieties of sugar beets, particularly for the hot lower zones.

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ON THE FORMATION AND EFFICIENCY OF PHOTOSYNTHETIC APPARATUS OF AGRICULTURAL PLANTS DURING THE VEGETATIVE PERIOD

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In our previous studies [1-4] it was shown that the formation of high yields of different crops is subject to many general principles. One of the most important circumstances is that more or less similar biological yields are the bases for low, average, good, and high yields of different agricultural plants (Fig. 1). Thus, low economic yields of different plants (grains, tuber and root plants, the green mass of corn, etc.) correspond to biological yields of 10-30 centners/ha of the total dry biomass, average economic yields of the same crops to biological yields of 30-60 centners/ha, good to 60-100 centners/ha, high to 100-150 centners/ha, and very high to 150 and more centners/ha.

Such a community of principles for the yield of many crops, even those greatly differing from each other, is due to the fact that biocoenoses (seedings, plantings), existing and developing on the basis of regularities common for them are the ultimate productive system; these regularities, to a considerable degree, govern individual characteristics of different plants, since biocoenoses similar in a number of important characteristics are developed as the result of different densities of seedings and plantings of different plants. In these cases different crops with different rationally selected densities of the plant stand develop under similar conditions a leaf area more or less identical with respect to rate of growth and dimensions; this leaf area is characterized by more or less identical indices of the rate and efficiency of photosynthesis and, consequently, fulfills more or less identical total work with respect to the formation and accumulation of the over-all biomass of the yields.

At the same time the growth and dimensions of the leaf area and the efficiency of their work depend very greatly on the environment. Thus, for example, with a good water supply and mineral nourishment in crops of different plants a leaf area can develop which reaches the optimum values (about 40-50,000 m^2/ha). Such crops can be characterized by indices of photosynthetic potentials of 2.5-3.0 million m^2 · days [4, 6], the net photosynthetic productivity of the leaves on the average for the growing period can correspond to 5-6 g/m^2 per day, and the biological yields can thus reach 120-180 centners/ha (for example, $3 \cdot 10^6 \times 5 = 150 \times 10^6$ g or 150 centners/ha). Under conditions of a limited water supply or mineral nourishment the leaf area of the crops of the same plants with the same density of seedings and plantings can attain only 25-15,000 and in the worst cases 10-8,000 m^2/ha, the indices of the photosynthetic potentials can be only 1.0-0.5 million m^2 · days, the average indices of the net productivity of photosynthesis decreases to 4-5 g/m^2 per day and, correspondingly, the biological yields are 40 or even only 20 centners/ha.

Having established these general regularities and indices, we consider it necessary [4, 6] to define them more precisely and to render them more concretely applicable to various plants, types and purposes of their cultivation, and, finally, to various conditions, regions, and zones of their cultivation.

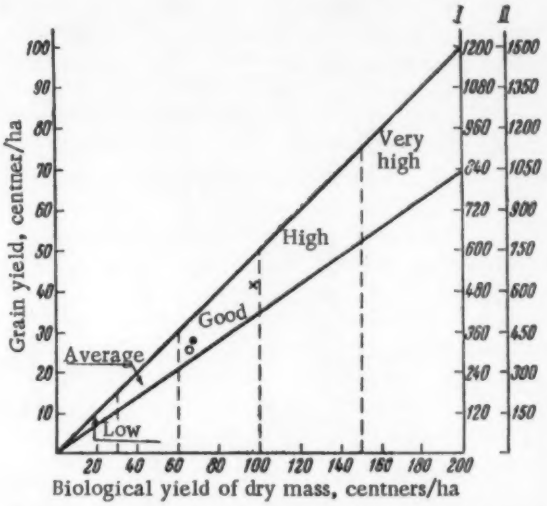

Fig. 1. Relation between the quantities of the biological and economic yields. Dry mass in centners/ha: I) root crops; II) corn. Tentative designations for 1959 economic yields: cross) corn with irrigation; solid dot) corn without irrigation; circle) spring wheat (grain).

One such problem requiring concrete expression is the following. Asserting that under similar cultivation conditions different plants produce economic yields on the basis of which lie similar biological yields, we nevertheless should bear in mind that different plants are sown at different periods of time, are characterized by a different length of the vegetative period, and, in conformity with this, even in the same region or during a single vegetative period, are subject during the course of growth and development in crops to the effect of different conditions and use them differently. Due to this, in a number of cases even in the same region of cultivation and in the same years, we can obtain yields from different plants greatly differing from one another, because during the course of growth and development inherent in them they use differently the conditions of the growing period. Thus, in a given region the most productive plants should be those plants whose course during the formation of yields most closely corresponds to the course of the agricultural meteorological conditions in a given zone and region.

In order to elucidate this problem we carried out a study in 1959 in a field of the scientific research institute for agriculture in central regions of the nonchernozem belt (All-Union Agricultural Scientific Research Institute, Nemchinovka Station, Moscow Oblast) on crops of spring wheat (Krasnozernaya variety) and irrigated and nonirrigated corn (Sterling variety).

In crops of these plants we took into consideration: 1) the variation in the growth of the leaf area; 2) the variation in the growth and weight gain during different time intervals of the total dry mass of the biological yields; 3) we determined on the basis of these data the indices of the net photosynthetic productivity; 4) we determined in addition to this the indices of the photosynthetic potentials of the crops and calculated the indices of the average photosynthetic productivity for each crop during the entire growing period. Less complete data were obtained for crops of sunflowers and a vetchoat mixture.

All the indicated indices were obtained by the methods described in earlier studies [1, 2, 6].

The crops subject to the study produced the following yields (in centners/ha).•

•We take the opportunity to extend our profound thanks to O. Ya. Gaak for making it possible to carry out the work on the crops indicated above, for his unvarying assistance in its accomplishment, and for submitting the data on the yield.

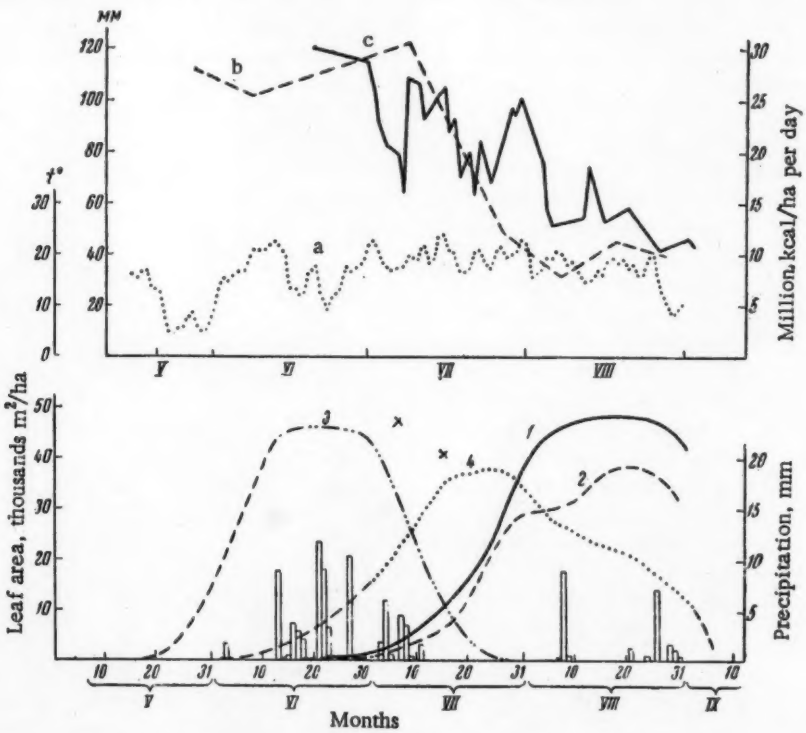

Fig. 2. Variation in the growth of the leaf area of corn with irrigation (1), corn without irrigation (2), spring wheat (3), sunflowers (4), vetch-oat mixture (×). In the upper section are the data of the variation of the mean diurnal temperatures (a), reserves of available moisture in a 50 centimeter layer of soil (b), and the daily amounts of the energy of solar radiation impinging on the crops (c). The columns are precipitation in mm.

Crop	Economic yield		Biological yield
Corn with irrigation	611	Dry mass	97
Corn without irrigation	412	for silage	66
Spring wheat	26.5 (Grain)		65

If we compare these yield data with the graphs (Fig. 1), we can state that in 1959 good yields were obtained in the crops indicated above. If we take into consideration the rather unfavorable conditions of the summer of 1959 (humid and cool first half, hot and dry second half), then it is necessary to consider the over-all result of the crops in 1959 as sufficiently good.

A graphic depiction of the variation in the elements for the formation of yields is given in Figs. 2, 3, and 4.

As to the environment during the growing period of 1959 (Fig. 2),† May and June were characterized by large reserves (100-120 mm) of available moisture in a 50 cm layer of soil, by a good radiation regime (daily amounts impinging on the crops of photosynthetically active radiation reached 30 million kcal/ha per day‡) and by frequent and abundant rains. Later we observed a gradual deterioration in the radiation regime, so that by the middle of August the indices fell to 10-14 kcal/ /ha per day. From the beginning of July the reserves of moisture in a 50 cm layer of soil were severely reduced: they declined from 100-120 mm in May-June to 40 mm in August.

In regard to the temperature regime we can indicate on the basis of the mean diurnal temperatures that May and, in part, June, were characterized by cool weather. In July the average temperatures were approximately 20°, which could have been favorable for the growth of the plants. However, the combination of such temperatures with the low July humidity created a strained hydrothermal regime. In August the temperatures began to drop, which could have been a somewhat unfavorable factor for the corn.

The course of the formational processes of the yield under these conditions was as follows. As is apparent from the data of Fig. 2, the maximum development of the leaf area in these plants falls in sharply different periods: in wheat it occurred in June, which coincided with the best radiation regime and high moisture content of the soil; in sunflowers it occurred in the middle of July whereby the subsequent decrease in the leaf area was undoubtedly accelerated by drought. The drought severely inhibited the rate of growth of the leaf area in the nonirrigated corn (Fig. 2, 2) and delayed the advent of the maximum index, which in the given case was observed only about August 20. Irrigation eliminated the delay in the growth of the leaf area in the irrigated variant of corn (Fig. 2, 1) and the indices of the leaf area for it already attained the maximum by about August 10.

Thus, if we make a general evaluation of the graphs showing the course of the development in the crops of the leaf area of different plants, we can say the following: wheat went through the growth cycle comparatively rapidly and in a period most favorable from the point of view of humidity and radiation. Corn, as a later crop, spent a considerable part of the first half of the growing season on the slow increment of the leaf area. The leaf area attained large values only by the end of July and beginning of August, at which time unfavorable soil moisture conditions developed, illumination conditions deteriorated, and, at the end, the temperature regime also became less favorable.

All this left its appropriate mark on the photosynthetic work of the leaf area of corn. Figure 3 shows the indices of the net productivity of photosynthesis for irrigated and nonirrigated corn. As is apparent, the highest indices of the net productivity of photosynthesis were detected during July, i.e., when the total leaf area of the plants was still small but its intensified growth was observed. By this same period of time, when the leaf area reached maximum values, the indices of photosynthetic efficiency were severely reduced and finally in August the larger leaf area of the corn, as a whole, worked inefficiently. This is seen in Fig. 4, where data are shown on the daily weight gains of the dry biomass (in kg/ha) during the growing season. It is apparent from the data in Fig. 4 that maximum daily weight gains of the biomass are observed not in the period of the maximum dimensions of leaf area, but in the period preceeding it, when

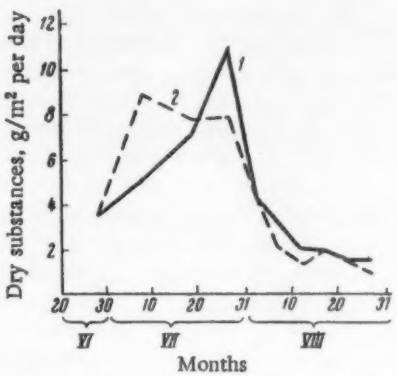

Fig. 3. Variation of the indices of the net productivity of photosynthesis of corn during the 1959 growing season. 1) Corn with irrigation; 2) corn without irrigation.

† The data on the reserves of available moisture in the soil and the variation of temperature and precipitation we received from the agricultural meteorological station of the Administration of the Hydrometeorological Service of the Central Oblasts, the Nemchinovka Station, and thus we are indebted and wish to extend our deep thanks to the workers of this station.

‡ The total amount of photosynthetically active radiation impinging on the crops was determined by S. N. Chmora by means of photointegrators [7].

the indices of net productivity of photosynthesis were highest.

The low efficiency of the work of the leaves in the period of their maximum development in the corn crops can be explained by the following causes: 1) by the unfavorable conditions of the water supply, which particularly severely affected the nonirrigated corn and the sunflowers; 2) by the deterioration in the conditions of the temperature regime, which is of vital importance for corn; 3) by the general deterioration in the radiation regime, which was still further spoiled by the fact that by this period the leaf areas in the crops had reached larger sizes and a strongly expressed mutual shading of the leaves must have occurred in the crops. In this respect the wheat crop was under more favorable conditions. Finally, it is necessary to note still another factor unfavorable for corn: in the wheat crop 4-6 million sprouts appeared from the start, which immediately (during the first days) developed on the whole a sufficiently large area. Thus, with a leaf area in each sprout of 2 cm^2 on the fifth day of their development, the total leaf area of the crop already by this time can be about 1000 m^2/ha. As to the corn, with a hill check of 60 x 60 or 70 x 70 cm with 2 - 3 plants to each hill there should be 40-100,000 sprouts per hectare. Even if we take that on the fifth day the leaf area of each plant is 20 cm^2, then in this case the total area of the plants per hectare on the fifth day is in all only 80-200 m^2/ha. With such magnitudes of the initial leaf areas and with doubling of the leaf area during each following five days, the leaf area in the wheat crop can reach 10,000 m^2/ha on the twentieth day (which corresponds to an optimum rate of growth). In regard to the leaf area in the corn crops, with the indicated initial magnitude it can reach dimensions of 10,000 m^2/ha on the twentieth day only by quadrupling every five days. However, the growth ratio of the sprouts of the wheat and corn plants in the initial period do not differ so severely.

Thus, with the above-indicated densities of the crops the total leaf area of the plants in the corn crops (especially late-ripening varieties) increases considerably more slowly than in the wheat crops. As a final result, in the described crops of 1959 the corn spent a considerable part of the growing season on the growth of a more or less considerable leaf area per hectare of crop and when the leaf area reached magnitudes which under other conditions could have been optimum the illumination conditions significantly deteriorated, which in combination with the strong development of the leaf area and the deterioration of the conditions of the moisture and temperature regime caused the photosynthetic and over-all productivity of the corn leaves to decline severely (Figs. 3 and 4). Continuing the comparison of the variations and results of the formation of corn and wheat yields, we can also note the following. The growing season for wheat was shorter (75 days) than for corn (85 days), but due to the more rapid increment of the leaf area the photosynthetic potential of the wheat crop was characterized by approximately the same index (1.94 million m^2 · days) as the irrigated corn (1.99 million m^2 · days).

As a final result the wheat and the irrigated corn produced almost identical biological yields, but the wheat did it in a shorter period of time, having used more favorable conditions of moisture and light. However, the wheat yield could have been even higher if a late-ripening variety had been in the crop which could have also used the favorable conditions of July. In regard to the corn, in the given case the potential possibilities of this crop were far from completely used. In order to cultivate corn more successfully in the central zone it is necessary: 1) to develop a system of agricultural technology such that it would be possible to show earlier sprouts; 2) the crops should be more densely planted than the present hill checked crops so that the number of sprouts reaches more than 100,000; 3) at the same time a system of mechanized handling of the crops should be developed such that in conjunction with herbicides it would assure a high economy in the crop; 4) varieties which ripen more rapidly than the Sterling variety and which are characterized by the fastest possible growth of the plants, especially in the early stages, should be used in the crops. As a result of all these measures the rate of plant growth should be such that the maximum development of the leaf area in the crops is reached at least in July, when the temperature and illumination conditions are still completely favorable and when the largest leaf area could work most efficiently.

At the same time, we will point out that in the 1959 crops, due to the severe decrease in the indices of the productivity of corn photosynthesis in August, when it was

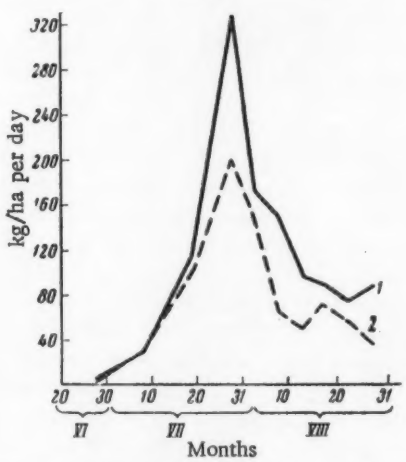

Fig. 4. Daily weight gains of the dry mass of corn during the 1959 growing season. 1) Corn with irrigation; 2) corn without irrigation.

only 2 g/m^2 per day, the over-all average indices of the net productivity were comparatively low for the entire growing period: for the irrigated corn the index was 4.86 g/m^2 per day; for the nonirrigated corn, 4.3 g/m^2 per day. At the same time, in the southern regions the average indices of net productivity of photosynthesis in corn crops were frequently 7-8 g/m^2 per day. We point out that during the 1959 growing season the index of net productivity for wheat was 3.4 g/m^2 per day, which also cannot be acknowledged as high and which was due, probably, to the illumination regime being somewhat lower than what is possible for this region in conjunction with the large leaf area produced in the crop with high humidification.

Thus, one of the tasks in the studies for increasing the plant yield under conditions of the nonchernozem belt should be to ascertain whether it is possible to increase the productivity of the photosynthetic apparatus of plants and to develop methods for this increase.

In conclusion we will evaluate the indices of the leaf areas which were obtained in the 1959 crops. At one time, when we started the investigation on developing a theory for producing high yields, we obtained materials on the basis of which we could consider that the optimum leaf areas in valuable crops should be 30-40,000 m^2/ha, although we indicated that this problem should be investigated further. As the investigations and the number of studied plants expanded, materials were obtained which testified to the fact that yields can be increased in those cases when the leaf areas at the maximum period reach 45,000 and even 50,000 m^2/ha [5].

Similar materials were also obtained in the 1959 study when irrigation of corn provided both the development of a large (over 40,000 m^2/ha) leaf area in the crop in comparison with nonirrigated and a higher yield.

In regard to wheat, due to the absence of a variant for comparison we cannot say whether in the given case a leaf area of 45,000 m^2/ha is optimum. We can only note that with such a growth rate of the leaf area only a good yield was produced at a comparatively low index of the net productivity of photosynthesis (3.4 g/m^2 per day in all), while in the wheat crops of the southern regions high yields (30 centners/ha of grain and higher) were produced at low indices of the leaf area and photosynthetic potentials of the plants, but with a net productivity of 7-8 and even 10 g/m^2 per day.

Thus, we can again emphasize that the problem of the optimum leaf areas of plants in crops is complex and requires further refinement. It is already possible to indicate now that a leaf area of 30-40,000 m^2/ha cannot positively be considered optimum for all crops and cases. An increase in these indices in a number of cases (for example, in a corn crop) to 50,000 m^2/ha and perhaps even higher can serve as one of the important conditions in increasing the yield. However, the obtainment of such an index is not always an easy task: good conditions of soil fertility and water supply are a must for this. In regards to the fields of the VNIIZ (All-Union Agricultural Scientific Research Institute), the level of the fertility of its soils and agricultural technology make it possible to obtain crops with a leaf area to 40-50,000 m^2/ha. This, however, is possible only if a good water supply is provided the crops, as is indicated by the wheat and irrigated corn crops.

How serious this factor is even in the central zone of the USSR is shown by a comparison of the irrigated crops and the nonirrigated crops of corn and sunflowers. However, it is necessary to note that in the nonirrigated crops the leaf area at the maximum period reached almost 40,000 m^2/ha. All this testifies to the fact that under conditions of the middle belt of the USSR on soils similar to those which are in the fields of the VNIIZ, possibilities exist for producing crops whose leaf area can reach 40-50,000 m^2/ha, which corresponds to the optimum index and is one of the important stipulations for obtaining high yields, on the base of which lie biological yields of 120-150-180 centners/ha of the total dry mass (see Fig. 1). Actually, however, in the fields of the VNIIZ in 1959 yields not quite so high were obtained. What is the reason for this? We already pointed out above one of them: the comparatively low indices of the net productivity of photosynthesis of the leaves, especially in those periods when the over-all area of the leaves in the crops reached high indices.

A second reason lies in the shortness of the active growing period of the plants: for the wheat it was 75 days and for the corn 85 days. Due to this, in spite of the fact that the over-all area of the leaves in the crops reached large magnitudes, nevertheless the photosynthetic potentials of the crops were comparatively small, being 1.75-2.0 million m^2·days. Here the wheat did not utilize the time which was still suitable for growing in July-August and the corn poorly used the conditions of June and a considerable part of July, and its growth was disrupted by a change in the weather conditions at the beginning of September. At the same time, if we cultivated wheat varieties having a longer growing period or earlier corn varieties it could have been possible to produce crops with a long growing season of 90-100 days, and the indices of the photosynthetic potentials could have corresponded to 2.5 million m^2·days [4]. In this case, even with an average productivity of photosynthesis in the crops of only 5 g/m^2 per day, such crops could have produced biological yields of 125 centners/ha, which corresponds to a high economic yield (see Fig. 1).

The selection of plants and varieties which would more completely and better utilize the conditions of the growing season is important for obtaining the indicated yields. This especially refers to crops whose cultivation requires planting of earlier varieties with the earliest possible planting periods, with densities of the plant stand somewhat greater than that used now, and with the fast-

est possible growth of the leaf area during the early periods. It is necessary that the leaf area of these varieties reach the maximum development in July-August in order that the active growth continue to the middle of August. We can obtain a positive result for wheat by cultivating varieties with a growing season longer than the Krasnozernaya variety since the latter does not utilize the favorable conditions of July-August. Under all conditions it is necessary that efforts be directed so that the early spring period when the moisture and light conditions are very favorable is used advantageously to the maximum.

SUMMARY

1. We investigated the course and results of the formation of yields in different agricultural crops from the point of view of the time and rate of growth, dimensions, and efficiency of the work of the photosynthesizing apparatus of plants—the total area of their leaves in the crops.

2. With a good provision of moisture the leaf area in wheat and corn crop attained 50,000 m^2/ha, which can, with other good indices, assure the production of high yields in conformity with the classification shown in Fig. 1.

However, the low indices of the net productivity of photosynthesis, the shortness of the active growth periods and their unsuitable distribution in time (especially for corn), as well as the low indices of the photosynthetic potentials of the crops, limited the possibility of obtaining completely high yields (120-150 centners/ha of the total dry biomass).

3. The process of the formation of the leaf area and yield for spring wheat is confined to the most favorable period of the season with the best moisture conditions and illumination regime. However, growth of spring wheat is completed in 75 days and a part of the period when the conditions are still favorable for growing (July) is not utilized by the wheat.

An increase in the yield of wheat under conditions of the nonchernozem belt is possible with the use of varieties having a longer growing season and higher photosynthetic activity.

4. In corn crops (Sterling variety) with hill checked planting, much time (more than a month) was spent before the total leaf area attained more or less substantial magnitudes (about 10,000 m^2/ha). Prior to this, due to the small area of the leaves, the corn crops could not operate with high efficiency and, thus, poorly utilized the best time of the growing season with good moisture and light. By the time the area of the leaves reached 45-50,000 m^2/ha the conditions of illumination, moisture, and shortly the temperature regime severely deteriorated. Thus, the leaf area which just reached full value (in August) operated with a low net productivity of photosynthesis (about 2 g/m^2 per day).

5. In order to increase the yield capacity of the green mass of corn in the central belt of the USSR it is necessary to strive for the earliest obtainment possible of sprouts with a larger number (not less than 100,000) of plants per hectare by improving agricultural technology and by developing appropriate varieties (earlier ripening, rapidly growing in the early phases), so that the optimum leaf area (40-50,000 m^2/ha) is formed in July.

6. Even in the zone of the nonchernozem belt of the central regions of the USSR the factor of inadequate moisture can be a condition limiting the attainment of high yields, and this requires serious attention.

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INVESTIGATIONS OF AEROBIC METABOLISM OF SUGARS AND AEROBIC RESPIRATION OF THE MERISTEM OF ROOTS*

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At the base of our concepts concerning the essence of life lies the notion of the need for a supply of energy to accomplish processes of vital activity. This energy is required not only for performing some type of work, but also in numerous metabolic syntheses. It is possible that the maintenance itself of labile submicroscopic structures of plasma also requires a constant expenditure of energy. One of the secrets of life is the automatism with which the release of energy occurs. This process, inherent to all life, we call respiration, which, in the end, is no other than the energy release of chemical compounds. There are a considerable number of such substances which can be used as the fuel or motor of life and at the same time there are different types of respiration releasing energy in a form which can satisfy the most diverse needs of a living organism. At the center of respiratory transformations in the majority of plants and animals are the sugars which are abundantly accumulated by plants during photosynthesis.

Our present-day concepts relative to normal respiration of higher plants are to a considerable degree based on the study of the processes of fermentation in lower, heterotrophic organisms. From the time of Wieland it has been known that in all biological oxidations, even in the presence of oxygen, the primary process is the splitting off of the hydrogen from the respiratory substrate. This fermentatively regulatable splitting off of the hydrogen at normal oxygen partial pressure then leads to the formation of water. With the use of sugars in the respiratory process the main portion of energy is thus supplied. However, another way is possible for the transfer of hydrogen split off from substances rich in energy. In the case when hydrogen is transferred to other organic compounds and is joined with them, such types of respiration occur which do not require oxygen and bear the name fermentations. These types of respiration are characterized by an energy yield lower than with oxidative respiration; the energy deficit arising in the case of fermentation is wiped out due to the intensified consumption of the substrate. Since with fermentation only a part of the hydrogen is split off, compounds even richer in energy—for example, alcohol—are formed as the end products. Anaerobic types of respiration are elements of aerobic respiration in which the original stages of cleavage can occur in the absence of oxygen. It is known that in the absence of oxygen the higher plants evolve CO_2 for a certain time, which indicates their ability to accomplish fermentation. This gives us the right to use data obtained in the study of fermentation of lower organisms for investigating the processes occurring in higher plants.

In our investigations we encountered the interesting fact that in certain cases uninjured higher plants with a normal oxygen partial pressure reveal both oxidative respiration and fermentation. During the course of the investigations, which included the measurement of respiration and the determination of the end products of fermentation, the presence of alcoholic fermentation in tissues of higher plants was ascertained [1].

The ways of aerobic and anaerobic breakdown of sugars have presently been studied rather profoundly. Under anaerobic conditions, as the result of conversions with the participation of fructose diphosphate, animal tissues form lactic acid, and yeast and higher plants form alcohol and carbon dioxide. Under aerobic conditions the carbohydrates are completely oxidized to water and carbon dioxide. Two means of oxidation are known to us: one is accomplished by the oxidation of glucose-1-phosphate and further conversions of the pentose cycle; the other means branches out at the stage of pyruvic acid and leads to the citric acid cycle. The degree of the participation of both means in the oxidative decomposition of carbohydrates has been inadequately studied and, of course, depends on the type of cells and tissues.

A successful study of respiration requires observation of both components of the gas exchange of tissue (CO_2 and O_2) and a simultaneous determination of the intermediate products. The starting point of our investigations was the works of Ruhland and Ullrich [2], who in the 1930's proved by means of microrespiration determinations the presence of so-called aerobic fermentation in meristematic tissues of plants. They detected in freshly cut

* The materials of this article were reported by Dr. Ramshorn on July 7, 1960 at the scientific conference at the K. A. Timiryazev Institute of Plant Physiology in Moscow.

growing parts a respiratory quotient higher than in differentiated tissues under the same conditions. This led them to the conclusion of the presence of intramolecular respiration in the plant meristems. It could not be completely eliminated even in an atmosphere of pure oxygen and therefore was given the name aerobic fermentation. Although we succeeded in confirming these data (with the exception of the behavior in pure oxygen), the heterogeneity of the tissues of the cambium, buds, and young leaves prevented strict conclusions. Therefore, in our further investigations we selected the root as the object. Roots, especially of sprouts, are easily divided into zones which correspond to individual stages of cell development, starting from the stage of division and elongation to differentiation of tissues. A shortcoming of this object is the radial differentiation, which, however, can be easily taken into account. In addition to this, studies with the root are especially very promising in virtue of the high specificity of biosynthesis of secondary substances in the root system.

An answer to the basic problem relative to the nature of the respiratory process peculiar to the tissues of the meristem is possible only after explaining certain systematic moments. An investigation of the gas exchange and conversions of certain metabolites in separate segments of the root cannot be carried out in situ on a whole undamaged root. This means that all analyses must be carried out on root segments within the shortest possible time. In order that the results obtained for the cut organs had some significance, it was necessary to confirm that during the experiment the physiological conditions of the cut-off parts at least were similar to those of an undamaged root.

We were able to answer the question of the comparability of the oxygen absorption by separate sections of the root and its segments by an electrochemical determination of the oxygen [3]. Oxygen absorption by an undamaged root was measured by means of an instrument based on a galvanic element consisting of a noble and non-noble metal. With correct operation of the instrument the current is determined only by the amount of oxygen which impinged on the noble metal electrode. With a uniform rate of flow of the investigated liquid (the buffer solution) the observed current is an exact measurement of the oxygen concentration in the given liquid. By means of this method we compared the oxygen absorption in situ by 12 sections of a root, each 1 mm long, with the gas exchange of corresponding cut-off sections of the root measured in a Warburg apparatus [4]. It turned out that oxygen absorption by various cut root sections (Q_{O_2}) remains almost unchanged for several hours after cutting. This justifies the use of data obtained in determining respiration of root segments for the characteristic of the corresponding section in situ. The investigation of the other gaseous component (Q_{CO_2}) cannot be carried out by an analogous method. However, Betz [5], who studied the exchange of ethanol in the root tips of peas, considers on the basis of his experiments that fermentation in pea roots is also not an artifact from preparation.

Our experiments, nevertheless, leave open the question of the correspondence between the manometric measurements of Q_{CO_2} and the existing conditions. We can be convinced of this by other experiments in which receptacles designed by us were used. Their use permits successive measurement of Q_{CO_2} and Q_{O_2} on the same material. This is accomplished by means of a large glass stopcock, the collar and core of which have abraded openings. In the empty inner space of the core is placed a strip of filter paper saturated with KOH, which by turning the stopcock can be connected or disconnected from the atmosphere of the receptacle.

In these and similar experiments we detected a different behavior in the cut root sections relative to the presence or absence of CO_2 in the atmosphere. Fixation of CO_2 activates the growth processes, although the absorption of oxygen remains unchanged. We will return to this problem later. In general we can consider as proved that the results obtained by the micromanometric method approximately reflect the gas exchange conditions in situ.

Our investigations of aerobic metabolism of sugars are based mainly on the study of gas exchange, on which we would like to dwell in more detail.

Figure 1 shows the data on the respiration of 12 1-millimeter pieces of radicles of Vicia faba, var. minor.

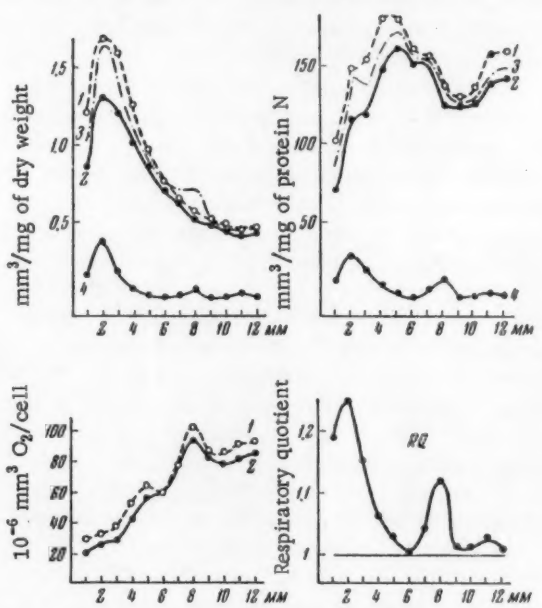

Fig. 1. Oxygen absorption by 1-mm root segments in one hour. 1) Q_{O_2} in pure oxygen; 2) Q_{O_2} in air; 3) liberation of CO_2; 4) excess liberation of CO_2. Plotted on the abscissa is the distance from the root tip in mm.

On conversion of the results to a unit of dry weight, the greatest intensity of respiration Q_{O_2} is detected in the root tips (from the first to the fourth millimeter). On conversion to protein nitrogen or to a single cell the ratios of the intensity of respiration of the separate sections of the root are substantially changed. However, regardless of the method of conversion the ratio CO_2/O_2 (RQ) remains reproducible. The root tip always displays an excess liberation of CO_2 which, according to our experiments, is caused by fermentation occurring simultaneously with oxidative respiration. The carbon dioxide excess gives a notion of the dimensions of fermentation, the type of which is established by means of special analyses. The formation of acetic acid is also possible along with the formation of ethanol [1]. The second maximum of the respiratory quotient indicating fermentation is detected 8 mm from the root tip. This approximately corresponds to the place of the tissue differentiation which is associated with processes of cell division.

These data are the average magnitudes obtained from numerous series of experiments during the first hours of measuring. They approximately correspond to conditions in the root in situ. For investigating the changes in time in the gas exchange of the cut root sections we first used the meristem since its respiration was especially characteristic. The most suitable for this purpose proved to be root tips 3 mm long, since the results of the rough calculation of the number of cells revealed the greatest number of cells in the first three 1-millimeter segments. Of course in the experiment we used those cells which displayed a tendency toward elongation. During the manometric investigations of the gas exchange of such root tips, we could detect during the first hours of the experiment a sharp decrease in the liberation of CO_2 at a comparatively constant absorption of oxygen. This continued until the Q_{CO_2} was somewhat lower than the Q_{O_2}. This process is shown in Fig. 2 by the left group of curves. The considerable excess of carbon dioxide completely disappears with time; the value of the RQ is correspondingly reduced to a value less than unity; in this case only oxidative respiration occurs.

The appreciable quantities of carbon dioxide being evolved during the first hours of the experiment are formed in the fermentation process and are not the result of the physicochemical retention of CO_2 by root tissues. This was shown in special experiments by Betz [6] and us [7]. Since the supply of the respiratory substrate does not occur to the separate root tips, we can assume that the reason for the disappearance of fermentation is the deficiency of the substances maintaining respiration. This assumption, expressed earlier by Ruhland and Ramshorn [1], was later confirmed in the investigations of Kandler [8]. This author showed that the respiratory quotient of 5-mm root tips of corn at first exceeds unity. If these rootlets do not receive a respiratory substrate from without, changes occur in their gas exchange. With the addition of 6% glucose the gas exchange remains unchanged, i.e., the fermentation rate is not lowered. We also attempted to prevent by means of a glucose solution the reduction of fermentation in our object—in the root meristem of beans. Numerous experiments showed that high concentrations of glucose inhibit oxygen absorption, and not in a single case did the addition of glucose maintain fermentation [7]. This is seen from the middle group of curves (Fig. 2). Therefore, at first we formed the opinion that the addition of a classical respiratory substrate (at first we tried only glucose) to the separate pieces of tissue does not eliminate the deficiency of the substances needed for cell functioning.

This made it expedient to study the endogenous, i.e., natural, sugars of bean roots (Fig. 3). The obtained results show that in the region of the root meristem where fermentation occurs, only sucrose is detected and glucose and fructose together with an insignificant amount of sucrose are contained in the basal parts of the root adjacent to it [9]. Glucose and fructose were detected for the first time together with sucrose at a distance of 4 mm from the tip of the root. It is known that the decomposition products of sugar render an inhibiting effect on its fermentative cleavage; therefore, it seemed probable that the addition of glucose to our object disrupted cleavage of the endogenous sucrose and its subsequent use in the processes of tissue metabolism. This also seemed to be indicated by the fact that the addition of an intermediate product of the anaerobic breakdown of sugar, pyruvic acid, for a long time maintains liberation of excessive CO_2 [7].

Pyruvic acid within definite concentrations intensifies gas exchange of the entire root section of beans stud-

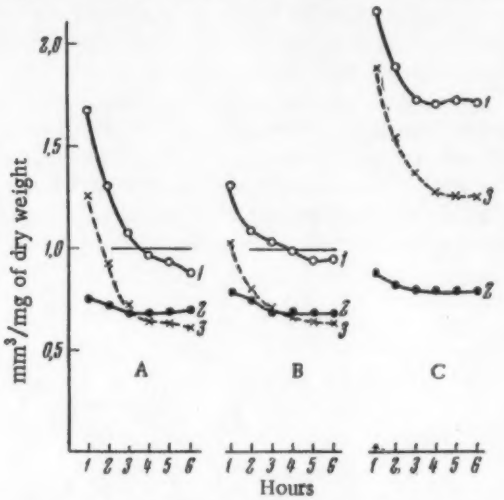

Fig. 2. Respiratory gas exchange of root tips with the addition of various substrates. 1) Respiratory quotient; 2) oxygen absorption in air; 3) liberation of CO_2; A) in moist air: B) in 1% glucose; C) in 0.118 M sodium pyruvate.

ied by us, whereby the liberation of CO_2 increases with the concentration of pyruvate, while the absorption of oxygen practically does not change. These results stimulated us to investigate the ability of our object to cleave glucose to pyruvic acid. This was accomplished several years ago in an experiment which should have proved the presence of fermentation.

As Holzer and associates showed, NH_4 ions can affect the triose phosphate dehydrogenase system of yeasts. At the same time it was shown that NH_4 ions affect syntheses connected with the consumption of ATP. The latter, according to Linen's theory, supply organic phosphorus and ADP for the triose phosphate dehydrogenase. A consequence of this is the intensification of the breakdown of sugars with an increased formation of pyruvic acid.

We added 0.05 M $(NH_4)_2 SO_4$ and detected in the course of the first hour a considerable increase in the formation of CO_2 at a relative unchanged absorption of oxygen, i.e., an intensifying of the fermentation processes [7]. This shows the possibility of experimentally accelerating the breakdown of glucose to pyruvate. On the other hand, the weak natural cleavage of glucose to pyruvate can be stopped by means of specific inhibition by iodoacetate. We succeeded by the addition of 0.001 M iodoacetate in suppressing the formation of excessive carbon dioxide, while the absorption of oxygen was only negligibly lowered. This selective stimulation or suppression of glucose breakdown to pyruvic acid is based on the specific activity or inhibition of the triose phosphate dehydrogenase system. This proves that anaerobic breakdown of carbohydrates, at least to pyruvic acid, follows the Embden-Meyerhof pathway. Betz [5, 10] showed an analogous fact for another object. In all these investigations the formation of excessive carbon dioxide served as the indicator of fermentation occurring along with oxidative respiration.

The question arises, however, why the accumulated pyruvate causes an intensification of the fermentation processes without intensifying the absorption of oxygen. This problem was quantitatively studied by Holzer and associates [11], who showed the presence of pyruvate oxidase and carboxylase in the mitochondria of yeasts. The Michaelis constant for pyruvate oxidase defining irreversible oxidation is by one order of magnitude lower than that of the carboxylase defining fermentation. It is clear from this that an increase in the pyruvate concentration above 10^{-3} m/liter intensifies only fermentation since pyruvate oxidase seems already to be supersaturated.

Thus, we conclude that glucose can only conditionally serve as a respiratory substrate of the root meristem of beans.

As a result of the earlier expressed considerations that glucose can prevent the consumption of endogenous sucrose, we compared the gas exchange of bean roots having additions of glucose, fructose, and sucrose. For this purpose we also measured the growth of the 3-mm root tips which we used. During the experiment it was possible to observe distinctly the increase of the rootlets, which we measured by means of a microprojector.

In our work, we, like Betz, used half-strength hydroponics solutions.

Glucose, fructose, and sucrose had different effects on the rate of gas exchange in air. Four percent solutions of all three sugars affected the absorption of oxygen. Sucrose showed itself to be the most acceptable respiratory substrate for bean roots. This was especially exhibited in air with a high content of oxygen [9]. Solutions of glucose, fructose, or a mixture of both sugars had less effect on respiratory gas exchange. This refers to the growth by elongation, which we measured after the roots were for 24 hours under the same conditions at which the gas exchange was studied. Sucrose has a stimulating effect on elongation, while the other two sugars inhibit growth. In these experiments we used only one concentration of the test sugars (4%); it is possible that the reason for the inhibiting action of glucose or fructose is the inhibition of cleavage of the endogenous sucrose by its breakdown products. Therefore, we investigated the effect of different concentrations of all three sugars on the respiration and growth both in air and in pure oxygen [9].

Here it was found that glucose has only a negligible effect on respiratory gas exchange; fructose and sucrose cause an increase in the respiratory quotient which in-

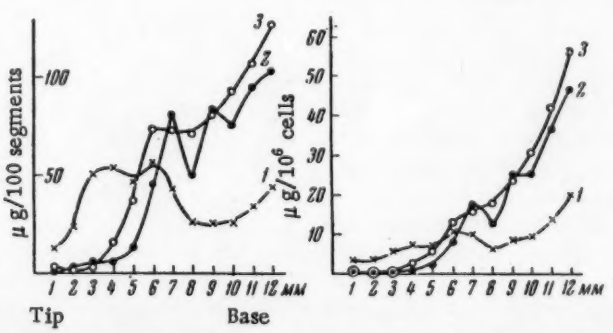

Fig. 3. The content of sucrose, fructose, and glucose from the tip of the root to the base. 1) Sucrose; 2) glucose; 3) fructose.

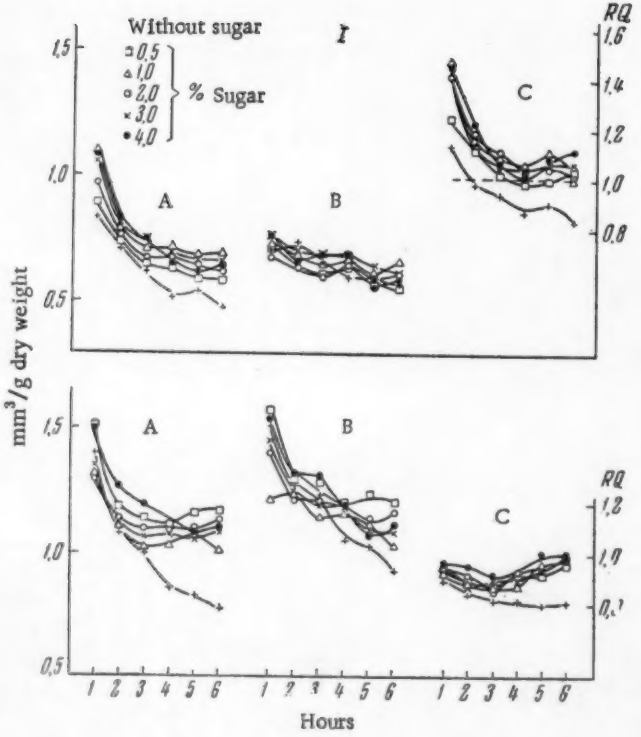

Fig. 4(I)

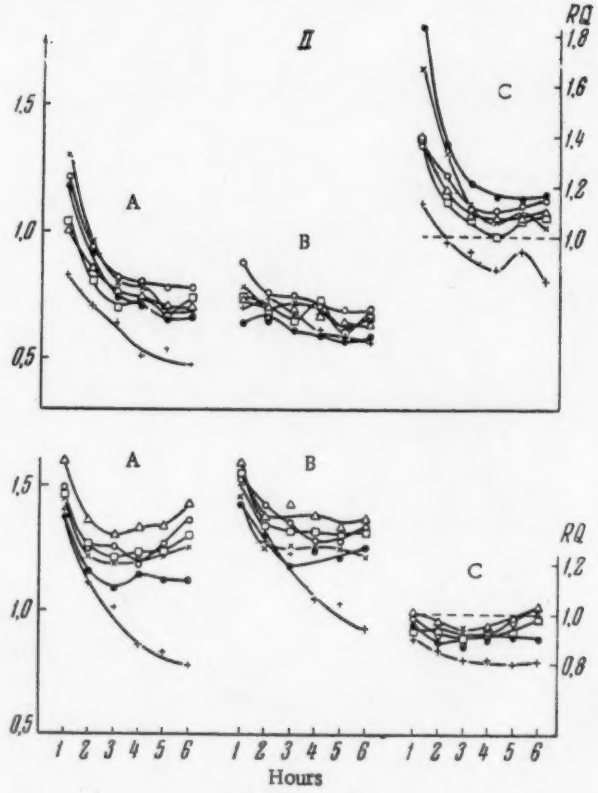

Fig. 4(II)

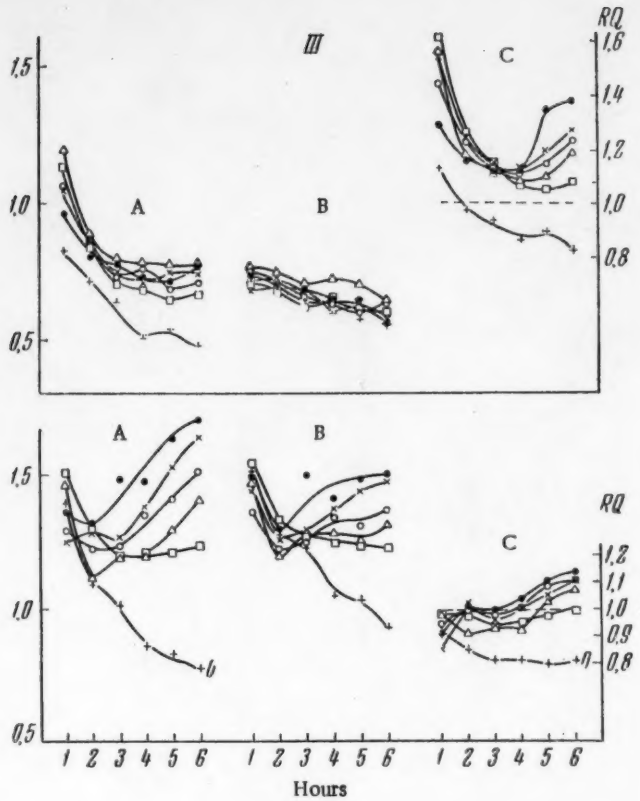

Fig. 4 (III)

Fig. 4. Respiratory gas exchange of the root with the addition of solutions of (I) glucose; (II) fructose; and (III) sucrose. A) CO_2 evolution; B) O_2 absorption; C) respiratory quotient. The upper portion is gas exchange in air; the lower portion is gas exchange in pure oxygen.

creases with an increase in the concentration of the solutions (Fig. 4). This is exhibited less strongly in pure oxygen. The effect of different sugars on growth by elongation bears a different character (Fig. 5). Sucrose and glucose cause maximum stimulation of growth in a 0.5% concentration; the optimum effect of the fructose is related to this same concentration but is considerably inferior in magnitude to the first two sugars. A higher concentration of sugars activates growth to a lesser degree or even inhibits it. An interesting result of these investigations is that within the optimum concentration all three sugars simultaneously increase both respiration and growth. A higher concentration has a different effect on respiration and on growth, i.e., in this case a direct relation between these two processes disappears. Thus, the 4% concentration of sugars which was used in our experiments is too high; it is not physiologic since it leads to a conversion of substances not comparable with processes in situ of interest to us.

However, an essential conclusion from these experiments is that in the meristem of bean roots glucose serves not so much as a respiratory substrate as a construction material in the growth process; fructose, apparently, is subject to cleavage to pyruvic acid. The amount of the latter is decisive for the appearance of processes of decarboxylation together with oxidation of pyruvate.

In the optimum concentration (0.5%) the glucose stimulates growth by elongation just as strongly as sucrose; however, the gas exchange under the effect of glucose varies less. Fructose, on the contrary, stimulates growth by elongation much more weakly. On the other hand, an equimolar mixture of glucose and fructose acts unlike sucrose. This indicates that the products of sucrose cleavage act differently than free glucose and fructose. It is probable, therefore, that in the process of respiration or carbohydrate metabolism primarily β-fructofuranose, or accordingly, glucose-1-phosphate are involved.

Support of this hypothesis can be seen in the fact that 0.125% pyruvic acid, while suppressing growth by elongation, intensifies decarboxylation [9]. Apparently the experimental increase in the level of pyruvic acid suppresses inversion of endogenous sucrose, which is the

main sugar of the meristem. As a consequence of this the formation of glucose-1-phosphate from sucrose is disrupted and growth by elongation is suppressed. Glucose and fructose in solutions of higher concentration can act in a similar manner, since the addition of these sugars leads to the displacement of sucrose from the enzyme-substrate complex during the cleavage process.

Due to the difference in the Michaelis constants of the enzymes of pyruvate oxidase and pyruvate carboxylase, fermentation sets in after complete saturation of respiration. Therefore the formation of excess CO_2 taken into account gasometrically in known limits reflects the dimensions of decarboxylation. However, on the basis of our measurements of the growth it is necessary to assume that the gasometric data do not sufficiently reflect fully the dimensions of the excess CO_2. If we examine the growth of root tips in the presence or in the absence of CO_2 we will come to the conclusion that the growth is related to carbon dioxide fixation [9]. The tips of the roots which do not receive CO_2 grow considerably less than in an atmosphere containing carbon dioxide. The values of the excess CO_2 which we measured are therefore underestimated by that amount of CO_2 which was used in fixation during the growth processes. Just like at the tips of the roots which do not receive sugar from without, fermentation processes continue longer than follows from the observed gas exchange.

The correctness of these notions could be checked by a quantitative determination of ethanol, which we carried out at the start of the experiment and after 6 hr of exposing the root tips under different conditions. The content of ethanol was determined by means of Warburg's optical method [12]. Similar investigations were carried out by Betz, who completely confirmed our data published in 1938. First of all, the curve of the alcohol content in 1 mm of the root tips in situ is very similar to the respiration curve (Fig. 6); the maxima of these curves coincide. The greatest amount of ethanol is contained in the meristems of the root tips. However, the high content of ethanol not only at the site of its formation but also in the zone of growth by elongation is surprising. The impression is created that the ethanol which entered the adjacent zones is not subjected to a further transformation. However, on conversion to a unit of dry weight of the different segments of roots we can note a sharp decrease in the ethanol content toward the base of the root. This indicates oxidation of the ethanol in those cells which are best supplied with oxygen. The possibility of oxidizing ethanol with an imptovement in the oxygen regime was shown by Betz [10]. The dimensions of this process under an ordinary atmosphere still require study. It is possible that low values of the RQ of those segments in which growth and differentiation occur are related to it.

The results of the experiments on the many-hour effect of exogenous sugars on the formation of ethanol are presented in Fig. 7. The group of columns on the left show the formation of ethanol after 6 hr exposure in the presence of 1% glucose (3), fructose (4), and sucrose (5). The amount of ethanol formed in the presence of sugars

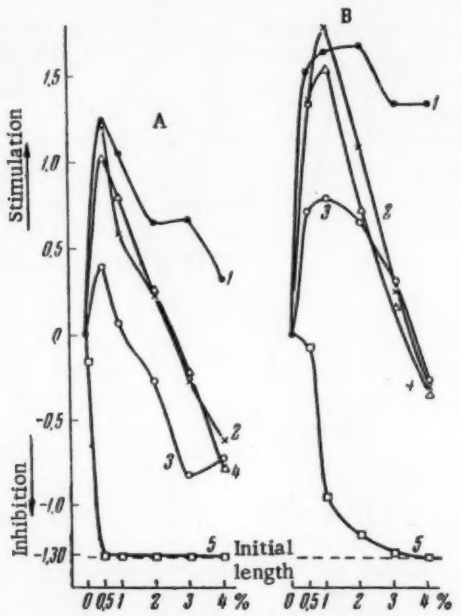

Fig. 5. The effect of sugars on the growth of roots by elongation. A) In air; B) in oxygen-enriched atmosphere. 1) Sucrose; 2) glucose; 3) fructose; 4) glucose + fructose; 5) pyruvic acid.

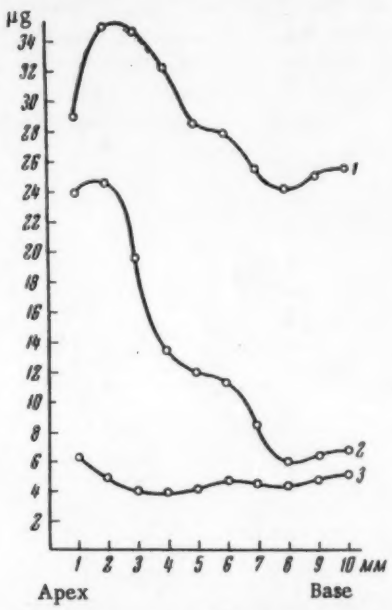

Fig. 6. Content of ethanol in seedling roots of Vicia faba from the apex to the base. 1) Per 100 segments; 2) µg per 100 mg of dry weight; 3) µg per 10^6 cells.

proved to be, in conformity with the results of gas exchange, higher than when using only endogenous sugars. Suprisingly high is the content of alcohol in the root tips which did not receive sugars, since according to the data on gas exchange it was possible to expect lower values. This indicates considerable dimensions of CO_2 fixation in the growth processes, which makes the determination of fermentation by the manometric method sufficiently accurate.

It follows from the figure that an increase in the concentration of sugars leads to intensified fermentation, which is indicated by the group of columns on the right. Glucose and fructose in this case cause an appreciable increase in the ethanol content; 4% sucrose, however, does not yield such a result. This is explained by the fact that the sharply expressed growth by elongation in the presence of sucrose results in the formation of numerous, well-aerated cells in which a partial oxidation of the formed alcohol occurs. The consumption of alcohol is difficult to take into account; however, it is possible to assume with certainty that in the presence of 1% and 4% sucrose considerably more ethanol is formed than we take into consideration during the analysis. The amount of alcohol formed by the addition of glucose or fructose almost fully corresponds to that calculated with respect to the magnitude of gas exchange and growth by elongation. As is apparent from what has been stated, glucose is partially involved in respiration, which, however, does not negate its preeminent use in biosynthetic processes.

We also determined what amounts of sugars are absorbed by tissue from the external solution [15]. In this case it was possible to note an especially sharp increase of sucrose (Fig. 8) after 10-12 hr. As we already saw, the intensity of gas exchange here correspondingly is reduced.

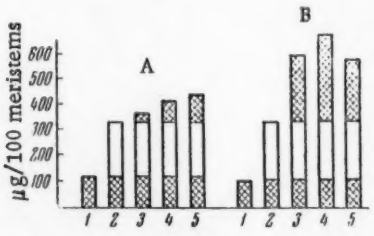

Fig. 7. The formation of ethanol in the meristem of a root tip in solutions of glucose, fructose, and sucrose. 1) Initial content; 2) after 6 hr in the medium without sugars; 3) glucose; 4) fructose; 5) sucrose. A) 1% solution; B) 4% solutions of sugars after 6 hr. The shaded part of the columns is the initial content of ethanol and the increase in the presence of sugars; the unshaded part is the formation of ethanol without addition of sugars.

We succeeded in exposing interesting regularities during the investigations of tissue sugars after 6 hr in 4% solutions of glucose, fructose, or, correspondingly, sucrose (Fig. 9). The additional sucrose nutrition leads to a certain increase of its content in roots; fructose has an analogous effect. Sucrose is not accumulated in particularly large amounts. Thus, the cells are penetrated not only by limited amounts of glucose and fructose, which could be expected, but also more high molecular weight sucrose was absorbed by the tissue. However, it remains vague whether the sucrose penetrates into the cells or is only adsorbed on their surface.

In the future the possibility of resynthesis of the sucrose inside the cell from its cleavage products should also be ascertained.

The results of these analyses support our notions of the character of respiratory transformations originating in the meristem of bean roots and also yield certain information on the initial and end products of fermentation.

The existence of alcoholic fermentation under aerobic conditions is proved; however, there is no single opinion on the causes engendering it [5]. On the basis of our data we postulate that it is caused by the difficult entrance of the oxygen into the compact meristem [9]. Under conditions of natural aeration and soil temperature, fermentation can be expressed still more vigorously than in the experiment. The problem of the further fate of the formed ethanol remains unresolved. On one hand it can be finally oxidized after translocation to other parts of the root, which assumes release of chemical energy mainly in well aerated parts of the plant. It is also possible that it is consumed in special syntheses about which we still have little information. This can

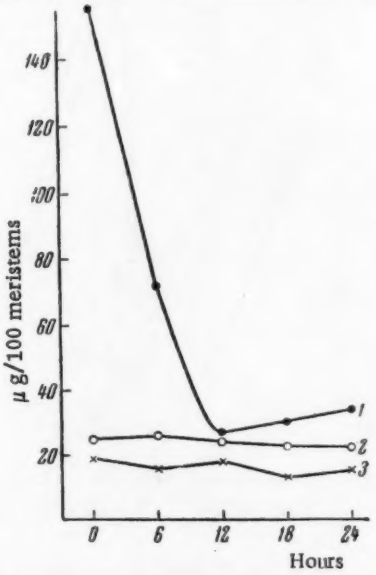

Fig. 8. Decrease of sugars in meristems of root tips of Vicia faba during 24 hr. 1) Sucrose; 2) fructose; 3) glucose.

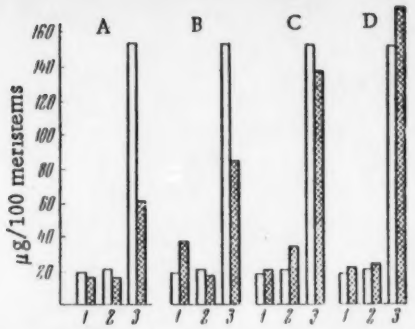

Fig. 9. The content of sugars in the meristem of root tips of Vicia faba in the absence (A); with the addition of 4% solutions of glucose (B); fructose (C); and sucrose (D). The unshaded columns are the initial content of sugars; the shaded are after 6 hr in an Edicott medium. 1) Glucose; 2) fructose; 3) sucrose.

be the main side of the physiological effect of ethanol on plants, about which Rieger and Michaelis [14] first reported.

An essential indication of the physiological effect of ethanol was recently made by another author. Kushner [15] succeeded in showing the effect of ethanol on the biosynthesis of enzymes which play a special role in metabolism. He proved that among several studied inferior alcohols, ethanol especially vigorously decreases the activity of lipase in Bacillus cereus. The impression is created here that the alcohol affects, not the activity of the already existent lipase, but its new formation during growth.

Betz was able to show that in an oxygen-enriched atmosphere ethanol is subject to a further oxidation by the meristem of the root, i.e., it is not only the end product of fermentation but also the substrate for further transformations. In addition to this we can assume with great probability that with the occurrence of fermentation the ratio DPN/DPNH is shifted to the side of DPNH. This should lead to a stable enrichment of the tissue by hydrogen and consequently to the reduction of SH-containing enzymes, which would favor the maintenance of their active state.

It is known that in the meristem of root tips the greater part of the SH-group is in a reduced and, consequently, active state [16]. The opinion of Betz is interesting, according to which reductive conditions and alcohol are in the meristem during fermentation, thus stabilizing the oxidation-reduction processes; however this requires experimental confirmation.

The notions cited here relative to the transformations of carbohydrates in the meristem are based on indirect proofs and concern mainly the initial and final phases of the anaerobic stage of fermentation. They can be considered only as the start of more profound investigations which we intend to devote to the study of enzymatic systems and intermediate products of metabolism.

SUMMARY

1. Under the conditions of the brief experiment, individual segments and sections of whole roots corresponding to them absorb oxygen with an identical rate.

2. In the meristem of the root there occurs, along with aerobic respiration, alcoholic fermentation which follows the Embden-Meyerhof pathway. The intensity of fermentation is determined by the rate of the formation of pyruvic acid.

3. In the meristem of the root tip of beans the glucose is largely used in the processes of biosynthesis; fructose is primarily subjected to oxidative decomposition. Sucrose is used in both these processes with considerably more intensity than the glucose, fructose, and their equimolar mixture.

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TEMPERATURE DEPENDENCE OF DORMANT SEED RESPIRATION

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1. Introduction

Numerous facts attest to the preeminent role of temperature in seed dormancy. Seeds of many plants must be stratified for long periods at low temperature (0-10 deg) before they will germinate. In warmth, such seeds, as a rule, do not germinate at all, or their germination capacity is reduced. Exposure of seeds to increased temperatures (from 20 to 30 deg) during stratification induces secondary dormancy.

The existence of a relationship between plant growth and respiration, and also the close dependence of both processes on temperature, compels the assumption that respiration plays an important role both during dormancy and during germination. Unfortunately the number of studies devoted to respiration of dormant seeds and of those undergoing stratification is extremely small. It is considered as established that respiration in the former is less rapid than in the latter [1-5], although some workers do not find such differences [6]. Data obtained in this and other [7-11] laboratories indicate a considerable influence of seed integuments on rate of gas exchange in dormant and stratified seeds. No qualitative differences in respiration of the two groups of seeds have been found [5, 6]. Values of the respiratory quotient (RQ) in dormant and germinating seeds obtained here and elsewhere [3-6, 11] are quite contradictory.

Respiration of seeds in a condition of secondary dormancy has been studied even less. At present, the view is widespread that this condition is a result of development of partial anaerobiosis [4, 12-14]. However, doubts have been expressed by Visser [11] and Nikolaeva, Kozlova, and Yudin [15] as to the anaerobic character of secondary dormancy in some plants. These divergent views are due in part to differences in methods employed, but are mainly attributable to experimental design. In most studies, measurements of respiration were carried out at room temperature—from 15 to 20 deg—or at higher temperatures—from 25 to 35 deg—in a water bath. For seeds which were being subjected to cold stratification, respiration was measured at a temperature to which they were wholly unaccustomed. Values obtained under such conditions were therefore indicative not of gas exchange of dormant seeds undergoing stratification, but of the after-effect of stratification temperature on respiration and the response of respiration to a change in temperature.

In order to clarify the nature of respiration of dormant seeds, careful studies of the temperature dependence of this process are necessary. This communication reports results of a study of the respiratory response of dormant seeds and those undergoing stratification to a change in temperature, this change occurring either at the time of measurement of respiration or at a preceding time. The seeds employed, those of Acer tataricum and Euonymus europaea, do not germinate in warmth, a lengthy period of cold stratification (4-5 months) being necessary to break dormancy [16, 17].

Measurements of respiratory rate were made in a circular Warburg apparatus [18]. Maple seeds were freed of pericarps and Euonymus seeds of outer coats, after which they were disinfected with 1% bromine water. Two or three replicate samples consisting of 40-60 seeds (2-3g net wt) each were used. Respiration was calculated on an hourly wet weight basis from data of two-hour readings. The method of determining RQ was varied with the type of experiment being performed. In studies of the effect of various temperatures on gas exchange, when it was necessary to make repeated determinations of RQ for a given seed sample, the direct method of Warburg was used. In other experiments, the method of Dickens and Simer was employed, which has the advantage that oxygen absorption and carbon dioxide evolution are determined in the same sample [19]. With both methods, RQ values are accurate to 0.1.

2. Seed Respiration at Various Temperatures

The temperature dependence of plant respiration has been investigated by many workers using a wide diversity of plant materials [20]. Information on the effect of temperature on respiration of dormant seeds is supplied by Harrington [3] and Visser [11] for apple, and by Brown [6] and Turkova [21] for oak.

In these experiments, respiration has been measured at temperatures from 3 to 35 deg. Measurements were made over a 2-hour period at each temperature, the seeds being maintained at a given temperature for 20 minutes prior to measurement.

Results of these determinations (Figs. 1 and 2) show that respiratory rates at low temperatures (from 3 to 10 deg) are very low, both in dormant seeds and in those being stratified, and that they rise with an increase in temperature. As a rule, respiration of partially stratified seeds was higher than that of dormant seeds. This was observed not only at 25 deg, but also over most of the temperature range investigated. The only exception to this was one of the Euonymus seed samples, in which the dormant seeds respired more rapidly than those being stratified. This sample was obtained from a different crop and weighed considerably less than the other samples, and thus the ratio of actively respiring embryo tissue to the much more sluggish endosperm tissue was higher.

Removal of the pericarp from maple seeds had little effect on rate of respiration at low temperatures. With an increase in temperature such seeds showed a much more pronounced rise in respiratory activity than did those with pericarp intact. The ratio of respiratory rate in seeds lacking pericarps to that of seeds possessing it increases most strongly in the range from 3 to 20 deg (Table 1).

Q_{10} values for maple seeds calculated from experimental data and from extrapolated figures (extrapolated to 13 and 15 deg) were highest at low temperatures (from

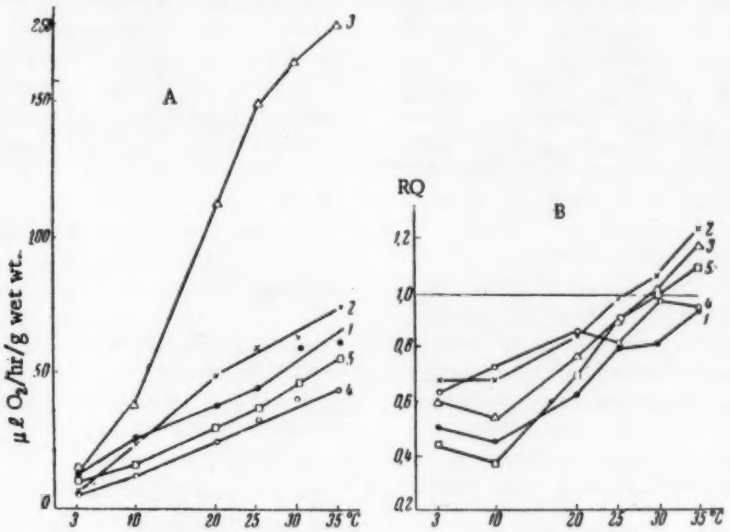

Fig. 1. Respiration of A. tatarica seeds at various temperatures. A) Rate of respiration; B) RQ; 1) seeds stratified at 0° to 3° 51 days; 2) the same, 80 days; 3) embryos stratified at 0° to 3° 88 days; 4) seeds stratified at 15° to 20° 51 days; 5) the same, 82 days.

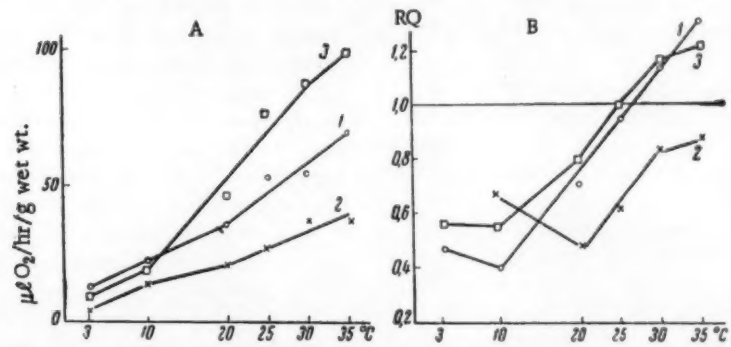

Fig. 2. Respiration of E. europaea seeds at various temperatures. A) Rate of respiration; B) RQ; 1) seeds (Kiev crop; wt. of 100 seeds, 7 g) stratified at 15° to 20° three months and at 0° to 3° 75 days; 2) the same, stratified at 15° to 20° seven months; 3) seeds (Leningrad crop; wt. of 100 seeds, 4 g) stratified at 15° to 20° seven months.

TABLE 1. Ratio of Respiration in Maple Seeds Lacking a Pericarp to That in Intact Seeds at Various Temperatures

Stratification conditions	Temperature, deg C					
	3	10	20	25	30	35
80--88 days at 15—20°	0.9	1.5	2.9	3.3	3.6	3.7

TABLE 2. Q_{10} of Maple Seeds Stratified under Various Conditions

Stratification conditions		Condition of pericarp	Temperature, deg C				
no. of days	temperature, deg C		3—13	10—20	15—25	20—30	25—35
51	0—3	Intact	4.0	2.0	1.6	1.3	1.2
51	15—20	The same	2.5	2.1	1.8	1.6	1.3
80	0—3	» »	2.0	1.5	1.5	1.4	1.4
80	15—20	» »	2.0	1.8	1.6	1.6	1.5
82	0—3	Removed	4.4	2.4	2.0	1.9	1.5

TABLE 3. Average RQ Values of Seeds at Various Temperatures*

Species	No. of determinations	Temperature, deg C					
		3	10	20	25	30	35
A. tatarica	17	0.6	0.8	0.8	0.9	1.0	1.1
E. europaea	6	0.5	0.5	0.7	0.9	1.0	1.1

*Values calculated from data obtained in all experimental variants.

3 to 13 deg) and decreased as temperature increased (Table 2). According to James [20], a decrease in Q_{10} with increase in temperature has been observed in the most varied plant materials. The reduction of Q_{10} to 1.2-1.5 over the temperature range 25 to 35 deg may be regarded as a reflection of limitation by the rate of gaseous diffusion.

The respiratory quotient (RQ) of dormant seeds and stratified seeds at 3 and 10 deg is very low, averaging 0.6 for maple and 0.5 for Euonymus. It increases as temperature increases and reaches a value of 1.0-1.1 at 30 to 35 deg (Table 3). Similar observations have been made with apple seeds [3, 11] and oak acorns [6], and also with seeds, seedlings, fruits, and other parts of various plants [20].

These data show that RQ of seeds depends neither on the presence or absence of seed integuments nor on pre-sowing treatment (see Figs. 1 and 2). The lack of a connection between physiological condition of seeds and RQ has been noted in Euonymus seeds by Nikolaeva and Karklina [5]. This requires further investigation, since Visser [11] found that in apple seeds the integuments exert a clear effect on RQ.

3. Dependence of Seed Respiration at 25° on Preceding Temperature Regime

There are reports to the effect that the high RQ's and respiratory rates which are observed in seeds just after transfer from cold to 25-30 deg later decrease [3, 15]. Other workers have not observed such a reduction in respiratory rate [11]. Experiments were designed to determine the length of time over which the elevated respiration at 25 deg is maintained in stratified maple seeds, and also to assess the significance of duration of the preceding cold period.

In one series of experiments, seeds which had undergone a two-month cold stratification were transferred to 25 deg. At different intervals following transfer, samples were taken for measurement of respiration. Parallel measurements were made of seeds left in the cold and of seeds kept for the same length of time in damp surroundings at 15 to 20 deg. In all cases, readings were taken at 25 deg 2 hours after transfer of samples from the storage temperatures (Table 4). Examination of data obtained in this experiment and of those obtained in determinations of respiration at various temperatures (Figs. 1 and 2) affords convincing proof that transfer of dormant seeds from 20 to 25 deg has no marked effect on respiratory rate or on RQ. An increase of temperature from 3 to 25 deg is associated with a three-or fourfold increase in respiration of dormant seeds. Similar treatment of partially stratified seeds results in an initial six- to sevenfold increase in respiratory rate. Moreover, RQ rises to 1.1-1.2 in stratified seeds, which is somewhat higher than in dormant seeds. It is important to note that a similar rise in RQ is observed in stratified seeds lacking pericarps.

Results of further studies bear witness to the fact that the increase in respiration and RQ of partially stratified

TABLE 4. Respiration of Maple Seeds at 25° as it Depends on Stratification Temperature

No. of days exposed to given temperature			Respiration, µl/hr/g wet wt.	RQ
0—3°	15—20°	25°		
0	74	0	31±1	0.6±0
0	78	0	37±0	0.7±0
0	88	0	34±2	0.8±0
0	97	0	36±2	0.8±0.1
74	0	0	63±5	1.1±0.1
78	0	0	66±2	1.0±0.1
92	0	0	62±4	1.1±0
97*	0	0	68±3	1.2±0
120**	0	0	188	1.1
58	0	5	48±5	0.7±0.1
58	0	16	32±2	0.8±0.1
58	0	32	31±3	0.8±0

*Seeds at −12° for 1 hr prior to determination of respiration.

**Respiration of seeds lacking pericarps.

seeds which is attendant on their transfer to 25 deg is temporary in nature. Measurement of respiration after five days at 25 deg indicated that RQ during this period fell to the level characteristic of dormant seeds which had not been exposed to cold at all (0.7-0.8). The increase in rate of gas exchange is more stable; after the same period, respiration of stratified seeds was still somewhat higher than that of dormant seeds. After 16 days, however, it was no higher in stratified than in dormant seeds.

In order to elucidate the nature of the rise in respiration of partially stratified seeds, determinations of respiration of dormant seeds which had been exposed to low temperature for only short periods were made (Table 5). It is evident that exposure for several hours or even days to "stratification" temperatures (from 0 to 3 and from 5 to 10 deg) is insufficient to induce an increase in respiration and in RQ. At the same time, exposure to −12 deg for 1 hour with a subsequent transfer to 25° induced changes in respiration similar to those observed in seeds subjected to prolonged stratification.

4. On the Role of Respiration in Seed Dormancy

With consideration of the data obtained, certain conclusions as to the role of respiration in dormancy and germination of seeds are possible. It is understood that the mechanism of dormancy is not under consideration.

Fig. 3 is a schematic representation of seed respiration during dormancy and during stratification under various conditions; also shown are changes induced by disruption of the stratification process. At temperatures near 0°, at which preparation of dormant seeds for germination occurs, the rate of respiration is extremely low. Under these conditions the oxygen requirement sinks to a minimum, while solubility of oxygen in water is increased. It is natural to suppose that respiration would always be aerobic under such conditions. The low RQ's

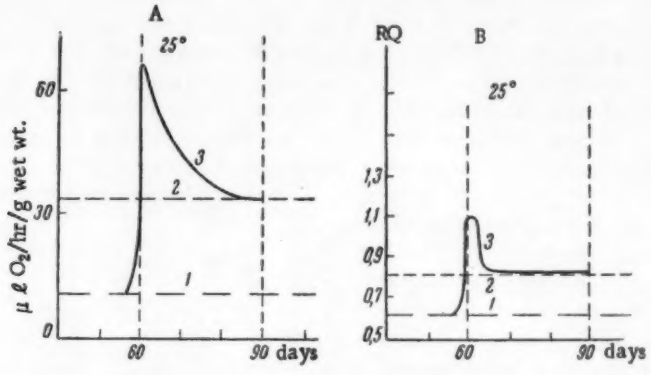

Fig. 3. Respiration of dormant seeds in relation to stratification conditions and also during induction of secondary dormancy. A) Rate of respiration; B) RQ; 1) average rates of respiration and RQs, measured at 3°, of seeds undergoing cold stratification; 2) the same, measured at 25°, of seeds undergoing stratification at 15° to 20°; 3) change in respiration with transfer of partially stratified seeds from 3° to 25° and exposure to the latter temperature for one month.

TABLE 5. Respiration of Maple Seeds at 25° after a Preliminary Short-Term Cooling

No. of days of preliminary exposure to given temperature			Condition of seeds	Cold exposure just before determination		Respiration, $\mu l\ O_2$/hr/g wet wt	RQ
0°-3°	15°-20°	25°		temp., °C	time, hr		
0	97	0	Primary dormancy	None	None	36 ± 2	0.8 ± 0.1
0	102	0	"	3-6	23	33 ± 2	0.9 ± 0
0	105	0	"	0-3	22	36 ± 2	0.8 ± 0
58	0	32	Secondary dormancy	None	None	31 ± 3	0.8 ± 0
58	0	44	"	3-6	23	36 ± 3	0.8 ± 0
58	0	44	"	0-3	20	39 ± 1	0.7 ± 0.1
58	0	38	"	0-3	192	41 ± 1	0.7 ± 0.1
58	0	36	"	0-3	144		
			Partial stratification	−12	1	57 ± 2	1.2 ± 0.1
92	0	0		None	None	62 ± 4	1.1 ± 0
97	0	0	"	−12	1	68 ± 3	1.2 ± 0

obtained in this and other laboratories support this assumption.

As is well known, hydrolytic processes are not impeded at low temperatures. At the same time, losses by respiration are extremely limited under these conditions. A situation is thereby created in which products of hydrolysis can accumulate gradually and in which there is apparently an accumulation of oxygen-rich compounds as a result of an aerobic metabolism. These changes, which occur in plant cells in the cold, promote growth processes and are critical in conditioning dormant seeds to a state at which germination takes place.

At temperatures above those at which stratification proceeds (above 10°), respiration is markedly enhanced and RQ increases. This is one of the reasons that hydrolysis products necessary to growth and emergence from dormancy fail to accumulate. If partially stratified seeds are transferred to higher temperatures, they undergo an abrupt rise in respiratory rate to a value almost twice that of dormant seeds at the same temperature. This inevitably entails a heightened utilization of hydrolysis products.

It is difficult to evaluate the increase in RQ which accompanies the increase in respiratory activity. As the data show, RQ is somewhat greater than 1 and might be regarded as an indication of anaerobiosis. However, earlier observations [15] throw doubt on this assumption. More likely, the increase in RQ indicates utilization in respiration of highly oxidized substrates, an assumption which finds additional support in the temporary character of the increase. Disappearance of accumulated reserves of highly oxidized hydrolysis products is probably the cause of the decline in RQ to a level characteristic of dormant seeds which have not undergone cold stratification. The rate of respiration falls somewhat later. The subsequent respiratory metabolism of seeds in a condition of secondary dormancy is no different from that of seeds in primary dormancy.

SUMMARY

The results reported, together with previously published information, provide convincing evidence that respiration of dormant seeds conforms to general biological patterns and lacks features specific to such seeds alone. This is particularly striking with respect to the effect of temperature, which plays a decisive role in the maintenance of dormancy and in its disruption. The response to changes in temperature of respiratory rate (Q_{10}) and of RQ is, in general, the same for dormant seeds, for seeds which have been undergoing cold stratification, and for non-dormant seeds.

The most clear-cut differences between dormant and stratified seeds which have been established to date have, in sum, amounted to higher respiratory rates in the latter. It is shown experimentally, however, that the increase in respiration which is observed in seeds stratified in the cold represents a temporary reaction to the higher temperature at which measurements are made. The respiratory rates of dormant and partially stratified seeds are shown graphically in Fig. 4. Reactions of plant and animal tissues to temperature change such as those illus-

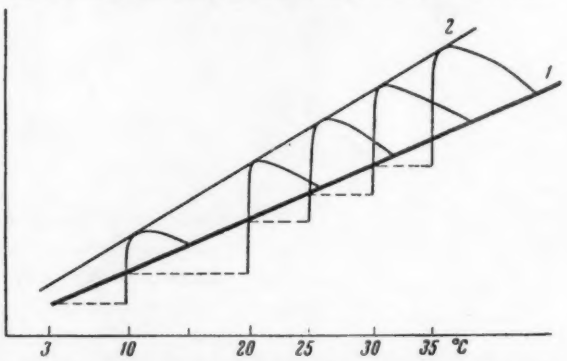

Fig. 4. Graph demonstrating differences in respiratory rate of dormant (1) and partially stratified (2) seeds at various temperatures.

trated in the figure are widely known, but they have not as yet been satisfactorily explained.

Sensitivity of a cell to a change in temperature depends on the species in question and also on physiological status of the cell. Stratified and dormant seeds differ sharply from each other in this respect. A change from 3° to 25°, or even smaller changes within this range, elicits in stratified seeds a temporary stimulation of respiration; dormant seeds show no response to such changes. Only if they were kept for an hour at −12° and then transferred to 25° could this response be induced. It is possible to find in studies of seed dormancy additional facts testifying to the higher sensitivity of seeds which had undergone cold stratification as compared with dormant seeds.

Obviously this study leaves much to be considered with respect to the role of respiratory metabolism in seed dormancy. It is necessary to clarify more fully the emergence and participation of anaerobic processes during induction of dormancy. Of great interest is the activity of various oxidative systems in relation to temperature, etc.

It should be emphasized that the above discussion pertains to seeds in which the dormant condition is related mainly to embryo status, which seeds require a lengthy cold stratification for their germination. As yet there is no basis for extending our conclusions to seeds in which dormancy depends primarily on properties of the seed integuments. The character of gas exchange in seeds of this type in relation to primary and secondary dormancy is a very pertinent subject for future investigations.

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EFFECT OF WATER SUPPLY ON THE ABSORBING ACTIVITY AND RESPIRATION RATE OF THE ROOT SYSTEM OF CORN*

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In previous studies [1] we have shown that preparatory, combined with growth, irrigation increased the physiological activity of the root system in summer wheat; the root bulk is increased as well as the total absorbing surface and particularly the active, working surface which accounts for the absorption of nutrient substances.

Corn was used for a further study of features in the root system's activity. We thought it would be of interest to trace the plants in environments of variable water supply with respect to development of their root system when it penetrates deeper into the soil, to changes in its absorbing activity in ontogenesis and, finally, to the roots' respiration rate, as one of the basic indicators of the organism's active life.

METHODS

The study was conducted under field conditions of the Rostov district on the Azov experimental field of irrigated agriculture of the Don Agricultural Scientific-Experimental Institute. The top soil of the experimental sector is a weak carbonaceous, medium clayey variety of pre-Caucasian black earth. The humus content of the tillable layer is 3.5%.

Experiments were conducted on two variants: without irrigation (control) and with irrigation, when the soil humidity was maintained at up to 70% of total field moisture capacity. Dimensions of the experimental plots—357 m^2. The experimental plant was corn, variety VIR-42. During the growing period two irrigations were conducted by filling furrows. The first irrigation was conducted on June 20 during leaf formation using 1100 m^3/hectare; the second on July 20 at the beginning of grain formation using 1200 m^3/hectare.

The planting was done on April 30 by a square-nest sowing machine. Before fall plowing this fertilizer was added: decayed manure 60 ton/hectare, superphosphate 3 centners/hectare and potassium salt 1.5 centners/hectare. In the spring (before planting) 5 ton per hectare was added of an organic-mineral mixture (compost plus superphosphate). During the growing period 3 additional feedings were given: the first—May 26 with ammonium sulphate 1.5 centners/hectare, i.e., 30 kg of active principle per hectare; the second—before the first irrigation, on June 12, also ammonium sulfate, 2.5 centners/hectare. The third additional feeding was conducted before the second irrigation (July 15)—by adding superphosphate, 1.5 centners/hectare, and potassium salt, 1 centner/hectare, i.e., 30 kg/hectare of the active principle of each.

For the study of the active absorbing surface we used Kolosov's [2] method developed under the leadership of D. A. Sabinin.

Soil monoliths with a root system, each 30 x 30 cm and 60 cm deep, were used, 15 cm distant from one another. Roots were washed and taken to the laboratory for analysis.

Sap was collected once a week from 6 plants of each variant. Receptacles for sap collection were adjusted on cut plants at 8 AM and removed at 1 PM, i.e., after 5 hours. The sap was measured from each plant individually.

The respiration rate of the root system was determined by Barcroft manometers in a water bath at 30°. Experimental roots were dug from a depth of 30 cm. All experiments were repeated twice.

Meteorological conditions in 1959 were typical of the Rostov district: an arid, hot summer. During the entire summer precipitation occured only 3 times. On such occasions the relative air humidity increased to 60%; the rest of the time it was low—30-40%. The month of July was especially hot and dry. The daily temperature frequently reached 30-35° with a low relative air humidity (below 20%) and dry winds.

Before consideration of our experimental data, it is necessary to note that the level of subsoil waters was located very close to the diurnal surface: at the beginning of growth at 70 cm, and only at the stage of grain ripening did it descend to a depth of 1 m and lower. This circumstance, as we shall see, definitely exerted an influence on the development of the root system and the entire plant.

*N. A. Fedorova, a student at Rostov University, participated in this study.

EXPERIMENTAL DATA

At the present time a great deal of attention is paid to the activity of the root systems. Abundant experimental material has been accumulated indicating that the plant roots are not merely organs of absorption of nutrient substances, but are also organs which perform synthesis of many organic compounds and play a large role in metabolism [3-9].

Literature sources [10-14] indicate that the greater the rate of growth and root development, the greater the rate of respiration processes and general metabolism, the more active is their absorptive activity, and the greater is the amount of nutrient substances furnished to the plant organs above the ground.

To determine the absorbing activity of the root system, as noted by Sabinin [3], Kolosov [15], Ratner [16], Krasovskaya [17] in their studies, knowledge of the magnitude of its active abosrbing surface is of great importance. It was especially important to trace how this magnitude varies in proportion to the penetration of the root system deeper into the soil, since we were interested in the interrelationship between the development of an active absorbing surface of the root system and its absorptive activity.

As seen from Fig. 1, the development of an actively absorbing root system's surface in corn plants during the growth period proceeded in a different manner as it penetrated deeper into the soil. However, no great difference was observed either in the control (nonirrigated) or in the irrigated plants in the initial stages of development, including the tassel formation stage. Evidently this was related to the provision of a necessary water supply to the root system of the nonirrigated sector at the expense of soil waters rising by capillarity to the upper soil levels and the active utilization of that water by young plant roots.

Only in the second period of plant development, in the stage of initial grain formation, did we observe a vigorous development of an active absorbing surface in the upper soil level with irrigated plants. This was caused by the fact that, beginning with the tassel formation stage, the weather was very hot with a daily temperature over 35°, low relative air humidity and dry winds. At this time additional nutrients and a second irrigation were administered to the irrigated sector, after which a massive growth of young, very active roots began in the upper level of 0-15 cm. At the same time the soil on the control sector at levels of 0-15 and 15-30 cm was dry; soil moisture was 30% of total field moisture capacity. The roots of control plants turned yellow, began to wither; their physiological activity was inhibited, which was evident by their marked reduction in sap secretion (Fig. 2).

The physiological activity of the root system cannot be characterized when only the magnitude of the absorbing surface is known. For this it is necessary to learn the absorbing activity of roots, i. e., the rate of absorption by a unit of this surface.

In order to characterize correctly the absorbing activity of roots—the development of an actively absorbing surface, at the expense of which the absorption of nutritive substances is accomplished—it should be compared with the amount of sap furnished by the root system to the plant organs above ground.

It is seen from Fig. 2 that the sap secretion during the growth period is not uniform and depends on the age, the plant's stage of development and the water supply. In the period of leaf formation, when an accelerated growth of the vegetative mass takes place, the quantity of secreted sap increases gradually in the control as well as in the irrigated plants. Toward the end of leaf formation it diminishes and again increases at the tasseling stage. A second sharp fall in the quantity of secreted sap in irrigated as well as in control plants is observed at the stage of tassel flowering, after which the control plants exhibit a further decline in sap secretion which

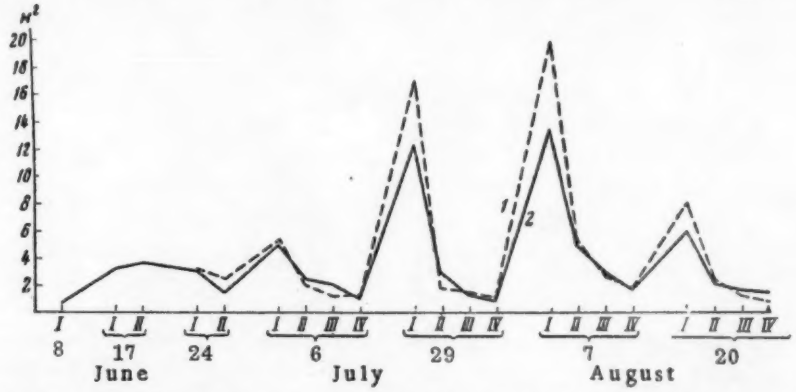

Fig. 1. Development of the actively absorbing surface of the corn root system with (1) and without (2) irrigation. Stages of plant development: from June 8 to July 24—leaf formation; July 6—tassel formation; July 29—grain formation; August 7—milky ripeness; I, II, III, IV—soil levels.

continues until the end of vegetation. However, after the second irrigation the irrigated plants again manifest a marked increase in the quantity of sap at the stage of the beginning of grain formation.

Thus, excluding the leaf formation stage, two maxima of sap secretion were observed in irrigated plants during the growth period: at the tasseling stage and at the stage of grain formation. Plants of the nonirrigated variant have no second maximum.

A similar phenomenon was also found in the studies by Petinov and Korshunova [18].

Having studied data on variations in the absorbing surface of the root system in plant ontogenesis and the amount of secreted sap, we can determine the absorbing activity of the corn's root system by comparing these two indexes.

By comparing curves in Figs. 1 and 2 we see that the absorptive activity of the root systems of nonirrigated as well as irrigated plants was alike at the beginning of the growth period, since the development of the root system's actively absorbing surface and the quantity of sap secretion hardly differed in the two variants.

During the tasseling period, notwithstanding the similarity in absorbing surface development and even its greater magnitude in the lower soil layers (at 15-30 cm and 30-45 cm) in nonirrigated plants, the bleeding saps were supplied in greater abundance to the above-ground organs of irrigated plants in comparison with the nonirrigated ones. At that time exceptionally hot and dry weather prevailed with a low relative air humidity, below 20%. The amount of sap in nonirrigated plants remained insignificant to the end of the growing period after it had fallen sharply during the flowering stage. But in plants of the irrigated variant a second maximum of sap secretion was noted at the period of grain formation. Hence, beginning with the tasseling stage, the absorptive capacity of the root system of nonirrigated plants, due to an atmospheric and soil drought in the upper soil layer, was inhibited to a large extent and was insignificant compared to the irrigated plants.

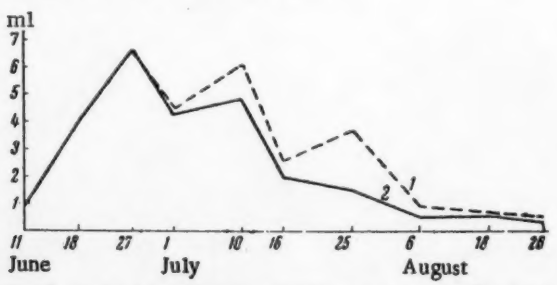

Fig. 2. Quantity of sap per single corn plant (ml/hr) with (1) and without (2) irrigation. Stages of plant development: from June 11 to July 1—leaf formation; July 10—tassel formation; July 16—tassel flowering; July 25—grain formation; from August 6 to August 18—milky ripeness; August 26—milky-waxy ripeness.

Thus, despite the adequate water supply of the lower soil levels and an almost equal development of the root systems in them, except for the upper level, their absorbing capacity differed greatly in the experimental variants. That is, when irrigated, the absorbing acitivity of roots beginning with the tasseling stage up to the end of growth was 2 to 3 times higher compared to nonirrigated plants. This circumstance was reflected in the extraction of nutrient substances from the soil as well as the accumulation of organic mass: 100 centners/hectare was the yield from a control nonirrigated sector, while 135 centners/hectare was the yield from an irrigated sector.

Since absorption of water and nutrient substances by the root system is accomplished at the expense of energy supplied as a result of respiration, determinations of the root system's rates of respiration were conducted for a deeper understanding of its physiological activity.

There is a great deal of material in the literature related to the study of respiration intensity, chiefly that of the leaf apparatus in various external environments [19-21 and others]. But there are relatively few studies devoted to the examination of the root system's respiration in field environments. And most of these are devoted to the study of the role of oxygen as a respiration factor affecting the absorbing function of roots [23-25].

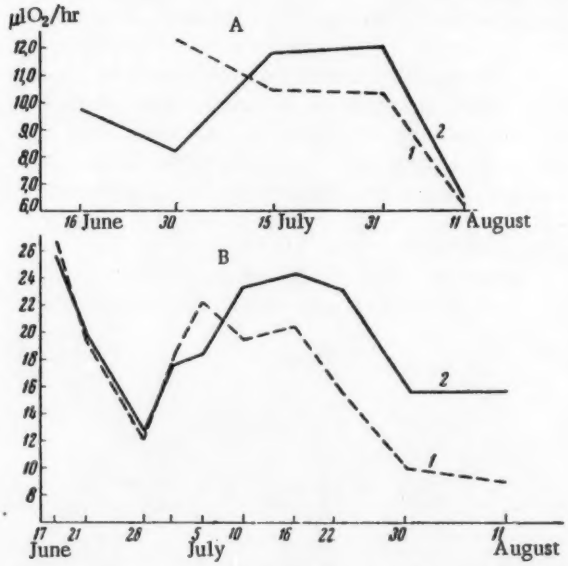

Fig. 3. Respiration rate of corn's root system with (1) and without (2) irrigation. A) Experiment in 1959. Stages of plant development: from June 16 to June 30—leaf formation; July 15—tassel flowering; July 31—grain formation; August 11—milky ripeness. B) Experiment 1955. Stages of plant development: from June 17 to July 10—leaf formation; from July 16 to July 22—tassel formation; July 30—tassel flowering; August 11—grain ripening.

During ontogenesis the respiration of leaves as well as roots varies constantly. A study of the regularity of these variations presents one of the chief problems in the plant's vital activities.

From Fig. 3A it is seen that in the initial stages of plant development the root system's respiration was considerably higher in irrigated compared to nonirrigated plants. However, beginning with the tassel flowering stage, i.e., in the period of greatest tension of meteorological factors (high temperature and low relative air humidity), the root system respiration of nonirrigated plants compared to irrigated ones increased sharply and remained at quite a high level until the end of growth.

Similar data on respiration of the corn root system we found in 1955 at the Engels experimental station of the Saratov district (Fig. 3B). The meteorological data in 1955 were similar to the 1959 data.

It is seen from Fig. 3B that the high respiration rate of the root system in irrigated plants during the leaf formation period becomes somewhat lower at the tasseling and flowering stages. The opposite happens to control plants—at a period of unfavorable weather conditions the respiration rate of the root system increases sharply during these stages and remains almost unchanged until the end of vegetation.

The increase in respiration rate in nonirrigated plants was previously noted in studies by other authors [19,22]. Some of these assumed that this increased respiration is related to the waste of a large quantity of nutrient substances and a lessened photosynthesis productivity.

It was subsequently established in studies by Petinov and others [26,27], and Zholkevich [20,21] that in reality an increased respiration is noted in nonirrigated plants, unrelated to an increased metabolism and all synthetic processes, but, on the contrary, related to a retardation of these processes and an unproductive waste of energy in resisting unfavorable conditions of environment.

Yastrebov [28] also notes an increased respiration in plants located in unfavorable nutrient environments. The increased respiration in nonfertilized plants he ascribes to a great expenditure of energy by these plants in absorbing difficultly accessible nutrient substances.

Thus, the high respiration rate of the root system of nonirrigated plants in our experiments is unrelated to an increased physiological activity of the root system, but most probably is related to an expenditure of a great amount of energy used to absorb nutrient substances and water under conditions of severely strained meteorological factors.

SUMMARY

Compared to nonirrigated plants, corn plants cultivated under irrigation conditions exhibited a better development of the root system and especially its active absorbing surface. The difference was especially noticeable in the upper layers of the soil. The amount of bleeding sap excreted by the roots was 2 to 3 times greater during the vegetation period in irrigated plants than in the control (nonirrigated) plants. Two maxima in sap excretion were observed in the irrigated plants—during tasseling and seed formation. In the nonirrigated plants, on the other hand, after a sharp drop in the bleeding sap content during flowering of the tassels a further decrease continued up to the end of vegetation.

The absorbing power of the root system was not very different in the various types of experiments at the beginning of the vegetation period. Under conditions of atmosphere or soil drought the absorbing power of the roots of nonirrigated plants during tasseling and throughout the remaining vegetation period was inhibited to a very large extent and comprised a small fraction of that in the watered plants.

Respiration of the root system of irrigated plants in the reproductive period is lower than that in nonirrigated plants. A sharp increase in the respiration rate is observed in the latter during this period as a result of severity of meteorological factors. This apparently is connected with a large expenditure of energy under unfavorable conditions required to maintain the vital activity in the root system and in the plant as a whole.

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THE DORMANT PERIOD AND WINTER HARDINESS OF APPLE TREES IN WESTERN SIBERIA

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In the study of plant frost resistance and winter hardiness, the dormant period, when external signs of growth are absent and physiological functions are markedly depressed, is of great significance. In a dormant condition the plant withstands all unfavorable conditions, such as low winter temperatures, drought, and high summer temperatures. The concept of "dormancy" is itself a relative one, since during this period very complex, albeit slow and imperceptible, embryogenic processes are taking place [1] to ensure continuation of growth in the following year. Equally complex processes which create the necessary resistance to unfavorable conditions are also occurring. The condition of dormancy has arisen in response to unfavorable environment in the course of evolution of a given plant species.

It has been established by many workers [2-5 and others] that woody plants growing in a mild climate must, upon completion of the growing period, be exposed to a certain period of low, above-freezing temperatures before they can resume growth the following season. During this period, growth is taking place in the embryonic parts of the plant, and until it is completed the buds are unable to produce shoots. The duration of this period and the low temperature requirement varies from species to species.

By observing throughout the winter the capacity of plants to renew growth, Howard [6], Strausbaugh [7], and Poyarkova [8] showed that the more frost-resistant plants and varieties require more time for inception of bud growth. On the other hand, it has been found by Abramov [9], Sergeev [10], Tupitsyn [11], and Nesterov [12] that species and varieties which have evolved under harsh continental conditions resume growth considerably earlier under favorable conditions than do related non-resistant species and varieties from a mild climate. This explains frost killing of Siberian berry apple in the Caucasus and the Crimea, where winter thaws induce it to resume growth prematurely.

It would appear that there is an inherent contradiction in the inverse relation between duration of dormancy (non-growth of buds) and winter hardiness. This is only apparent, however, since the process of preparing a plant to renew growth is related to the environmental conditions which have prevailed during evolutionary formation of the given species. To be specific, plants from places with a harsh continental climate pass through the dormant period more quickly and at lower temperatures, while those from milder climates require more time and are capable of completing the dormant stage at higher temperatures.

From this it follows that a long lasting "non-growth" period cannot be associated with capacity to withstand low winter temperatures. Potapenko [3] has established that "emergence from dormancy (i.e., acquisition by buds of capacity to grow—A. Kh.) cannot reduce frost resistance to dangerous levels, since transition to the dormant state in summer does not signify assumption of winter frost resistance." This means that frost resistance is developed as the plant passes through the dormant period, and that it is not always related to the plant's capacity to resume growth.

Tumanov and Krasavtsev [13] and Kuz'min [14] have shown that in winter, with a reduction in temperature, frost resistance of woody plants and of grape rises, while toward spring, with the warming up period, it gradually falls. During dormancy, therefore, certain processes occur which promote an increase in this property.

It has been shown by many workers [8, 15-19] that during the dormant period complex and profound alterations in reserve nutrients take place in plant tissues. These have been traced with especial care for starch, sugars, and fats. About the middle of winter there is a marked decrease in starch and increase in sugars and fats in tissues of woody plants, with the tissues more resistant to frost containing more fats.

Genkel' [20] and Genkel' and Oknina [1,18,21] have established that in the dormant period there is a "balling" up" of the protoplasm and a decrease in its swelling capacity as a result of the formation of larger amounts of hydrophobic lipoidal compounds. This process is pro-

longed and reaches its most pronounced expression in the middle of the winter, i.e., at the time of the lowest temperatures.

This brief survey of studies of plant dormancy shows what complex changes occur during this period. In summary, they are as follows: At the end of the summer, when visible growth has ceased, a vigorous flow of nutrient reserves to the overwintering parts begins; with the onset of low above-freezing temperatures, embryonic growth in the buds occurs; and with the onset of below-freezing temperatures, complex conversions of reserve nutrients, rounding up of protoplasm, and change in its colloidal condition begin in preparation for withstanding the low temperatures.

It is thus apparent that in elucidation of frost resistance and winter hardiness, the study of winter dormancy is of considerable importance. Since this latter property depends on environmental conditions which prevailed during evolutionary formation of the species, and since it is an inherited property, it must be studied under various climatic conditions using plants with different genetic makeups.

In this study we have attempted to trace a number of physiological changes which occur in different varieties (species) of apple of the Novosibirsk area.

Apple trees in the orchards of Novosibirsk and environs are represented by three groups of varieties and by Siberian berry apple, which is used as a graft stock and for ornamental purposes. The first group is made up of varieties of rennet apples, which are closely related to Siberian berry apple (Malus pallasina Jur.). Their floral and vegetative buds and cambial tissues have a fairly high frost resistance and winter hardiness, and their wood is somewhat less frost resistant, though still fairly so in many varieties. The fruits are small (5-10g) and sour, and are utilized for commercial purposes. The second group is made up of semicultivated varieties (sometimes called "Chinas") which have been derived from Chinese apple (Malus prunifolia Willd) or, most commonly, from the hybridization of rennets with European varieties or with their progeny obtained by free pollination. The frost resistance and winter hardiness of buds, cambium, and wood is considerably lower than in rennets, so that they can grow successfully only in the southern regions of Siberia. Their fruits are larger (15-30 g) and of better taste quality. The third group comprises varieties from temperate Russia and Michurin large-fruited varieties, which do not possess sufficient frost resistance for Siberia, and when grown in upright form die back to the snow line. In order to obtain crops and to ensure survival of the trees, they are grown in recumbent form, so that the branches are covered with snow. The fourth group is represented by Siberian berry apple, which is highly frost resistant in all its aerial parts, but which produces very small fruits (less than 1 g) of extremely poor taste quality.

It is thus apparent that species and varieties of apple are extremely diverse with respect to their frost resistance and winter hardiness. In Siberia these are the critical features on which selective breeding of new varieties and introduction of established varieties from other places must be based. The present methods of diagnosis of frost resistance, which require a long time, make determination of the hardiness of new varieties a lengthy process.

By studying certain physiological processes occurring during winter dormancy in species and varieties of apple differing as to frost resistance, we have attempted to establish a regular relationship between such processes and frost resistance.

It was noted above that during dormancy a plant passes through various stages in which changes of a qualitative and quantitative nature occur. Johansen [22] has distinguished three stages: preliminary, intermediate, and final. Peretolchin [16] designates the intermediate stage as that of profound, true dormancy, and the final stage as one of "enforced inactivity." Molish [23] divides dormancy into obligate and enforced stages. Maksimov [24] divides it into three stages: preliminary, deep, and enforced. The main criterion for these divisions is the ability of buds to grow out under favorable conditions (as a rule, growth of branches in the laboratory). The stage of "deep" dormancy is that in which the plant is unable to resume growth. Many workers [8,9,11,16, 25] have attempted to establish an interdependence of frost resistance and the length of this stage, but information obtained is extremely contradictory.

In 1958-1959 and 1959-1960, we performed a study of "deep" dormancy in which behavior of buds on excised branches transferred to the laboratory and kept in water was observed. Every three or four days the water was changed and fresh branches were brought into the laboratory (extended method). Samples were collected for analysis every two weeks beginning in October. Varieties employed were: a) rennet group—Purple rennet, "Pudovshchiny"* seedling, Yantarka altaiskaya, Resistant; b) semi-cultivated group—Dolgoe, Ural juicy, Altai pepin, Yellow chaldon, Rosemarine startseva; c) European group—Juicy white, Pepin saffron, Slavyanka, Borovinka, Streifling; d) Siberian berry apple.

It was observed that buds of Siberian berry apple, the most resistant variety, began to grow out first (end of October—beginning of November), those of resistant rennet varieties somewhat later (last half of November—beginning of December), and those of the non-resistant European varieties still later (December). The semicultivated varieties fell between the rennets and the European varieties, with some variation in both directions. Capacity to grow was assessed according to behavior of vegetative buds and formation of new growth. Floral buds emerge from dormancy considerably earlier in all va-

* Transliteration of Russian — Publisher.

rieties under study. It was found that at the beginning of winter the first buds to grow were the terminal buds, which formed a rosette of leaves or a small shoot growth. A similar observation was made by Poplavskii [26]. We became interested in whether this was due to an absence of roots or whether various tissues emerge from "deep" dormancy at different times.

In order to obviate the difficulty inherent in the method (absence of roots), two-year-old slips of Siberian berry apple were set out in metal containers in the spring of 1959, and also one-year-old grafted seedlings of three varieties (Purple rennet, Dolgoe, Pepin saffron). During the summer the plants formed vigorous root systems and produced good growth. Beginning on October 15, banks of plants were brought into the laboratory (two plants in each group) every two weeks and gross observations were carefully made. These observations revealed that Siberian berry apple is in fact prepared to resume growth earlier than the other varieties, and on plants brought inside on October 15 there were already a few buds which had grown out, these being, again, the terminal buds. The other buds were still in a dormant condition. Plants brought in on October 31 had four terminal buds each which had opened; those brought in on November 16 had 5.5 buds, and those brought in on December 2 had 8 buds which had grown out (Fig. 1). Counts were made on 10 shoots of each of two plants a month after transfer to the laboratory. These revealed that buds emerge from dormancy in sequence from the shoot apex to its base. A similar tendency was observed in the other varieties under study (Purple rennet, Dolgoe, Pepin saffron).

Our observations permit us to conclude that in apple the vegetative buds undergo their embryonic growth not in synchrony, but in sequence, this process requiring quite a long time for its completion (in our experiment, three months). It is therefore impossible to determine the time of completion of the stage of "deep" dormancy by the behavior of terminal buds alone. This method must be

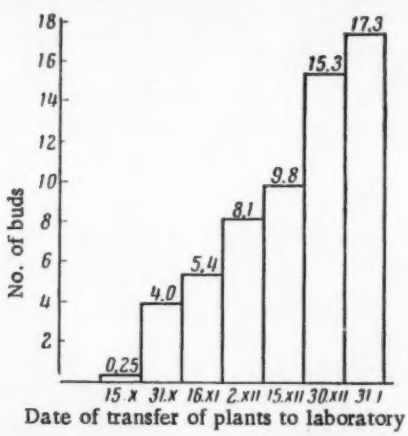

Fig. 1. Average number of buds which had grown out on a one-year-old growth of Siberian berry apple.

regarded as invalid, since it does not reveal when the plant is fully ready to resume growth.

Another conclusion may be drawn, namely, that embryonic growth processes in vegetative buds of apple can take place not only at low, above-freezing temperatures, but even at sub-freezing temperatures. This is shown by the fact that in Novosibirsk there was continuous frost from October 31, at which time the majority of buds of Siberian berry apple, and all the buds of the other three varieties, were dormant.

A study of the dynamics of starch and fat conversions, rounding up of protoplasm, and swelling of protoplasmic colloids was made with transverse sections taken from the lower regions of one year's growth. Sections were made with a hand microtome.

Changes in reserve nutrients were determined histochemically, using the iodine test (Lugol's solution) for starch, and scarlet red and Sudan III for fats. Amounts of these substances in the tissues were expressed in arbitrary units, starch according to the number of grains in cells and their disposition in tissues (method of D. F. Protsenko), and fats according to staining intensity, using previous analyses as a standard. In 1958-1959 Siberian berry apple and varieties Dolgoe and Juicy white were analyzed, and in 1959-1960 Purple rennet and Pepin saffron were analyzed as well. Analyses were made monthly from September to May.

Many workers [1,8,16,17,18,27,28] correlate degree and rate of starch conversion during the winter period with length of the "deep" dormancy stage and with frost resistance, i.e., the more rapidly and completely starch is hydrolyzed, the greater the plant's frost resistance and the more prolonged the period of "deep" dormancy.

Our observations are in agreement with the first of these assumptions with respect to frost resistance. Thus, in Siberian berry apple, the most resistant apple studied, we found total disappearance of starch in cells of the previous year's growth, while in the semicultivated variety Dolgoe some starch remained unhydrolyzed, and in the nonresistant variety, Juicy white, there was a considerable amount of starch present. This is clearly evident in Fig. 2A.

It is impossible, however, to concur with the statement that greater starch conversion in the course of a winter corresponds to a longer stage of "deep" dormancy (as determined by the growth of buds under favorable conditions). In the species (varieties) under consideration, the reverse is true: Siberian berry apple, which converts starch to the greatest extent, is capable of resuming growth before any of the other varieties, while variety Juicy white, the cells of which contain much starch in the winter, requires more time for the ripening of vegetative buds to a state in which they are able to produce new growth.

In every species (variety) examined, fats accumulate during dormancy predominantly in the cambial tissue, which has the highest frost resistance.

Conducting elements of the periderm and bast parenchyma, which are adjacent to the cambium, exhibit considerably less staining. In the least resistant tissues, xylem and pith, no staining reaction for fats was observed at any time during the winter. Considerably larger amounts of fats are accumulated in cells of Siberian berry apple than in those of varieties Dolgoe and Juicy white (Fig. 2B). This type of distribution of fats in tissues indicates that frost resistance is to a significant degree determined by extent of fat accumulation.

Genkel' [20,29] and Genkel' and Oknina [1,18,21] believe that the condition of dormancy is related to a rounding up or shrinking of cell protoplasm and a change in degree of colloidal swelling capacity (determined by time for appearance of cap plasmolysis in thiocyanate solutions). We have followed these processes with transverse sections from the last year's growth. Analyses were carried out according to the method of Genkel' and Oknina [18].

Rounding up of protoplasm was followed according to the form of plasmolysis of parenchyma cells in a molar solution of sucrose. In all species and varieties investigated, this was most pronounced in the middle of the winter, being uniformly of the convex type. With the advent of spring, the cells lose their capacity for protoplasmic shrinkage, with the less resistant species being first. In resistant varieties this shrinkage phenomenon is more pronounced than in nonresistant ones.

Parallel measurement of the time for appearance of cap plasmolysis in a molar solution of potassium thiocyanate was made in cells of the cortical parenchyma. It was found that plasmolysis time is greatest in Siberian berry apple, with the semicultivated variety Dolgoe having a somewhat shorter time and the little resistant variety Pepin saffron having a still shorter time. Considerably more time was required in winter than in autumn, and especially more than in spring.

Time of Appearance of Cap Plasmolysis in Cortical Parenchyma Cells of One-Year-Old Growth in Apple (in min)

Species (varieties)	Nov.-Dec.	Jan.-Feb.	April
Siberian berry	25-30	72-80	8-10
Dolgoe	14-19	40-50	3-4
Pepin saffron	7-10	18-21	2-3

It appears, thus, that during the period of dormancy there occurs a change in colloidal systems which leads to reduced swelling capacity in the coldest parts of the year. These changes are most profound in the most resistant species (varieties). These findings are similar to those of Genkel' [20] and Genkel' and Oknina [1,18,21] for apple varieties growing in the European section of the USSR.

The measurements of starch and fat content, and of rounding up and swelling of protoplams in cells of one-year-old growth, show that during the dormant period a very complex and profound reorganization occurs. This is related to changes in the surrounding environment in the following manner. With the advent of winter, as the temperature falls, there is a reorganization of the protoplasm, manifested as a more pronounced tendency to round up, and an increase in amounts of fats and lipoidal substances. Simultaneously, there is an increase in frost resistance. These alterations are most vividly apparent in the coldest part of the winter (January). With an increase in temperature toward spring, the reverse processes occur, these being concerned with the creation of favorable conditions for growth resumption. During this time there is a gradual reduction in tissue frost resistance.

As shown by many studies [1,18-21,25,29,30,31], a number of other physiological processes take place during dormancy; these are concerned with changes in protein metabolism and in levels of nucleic acids and oligosaccharides. This gives reason to believe that the delimitation of the dormant period into "deep" (organic)

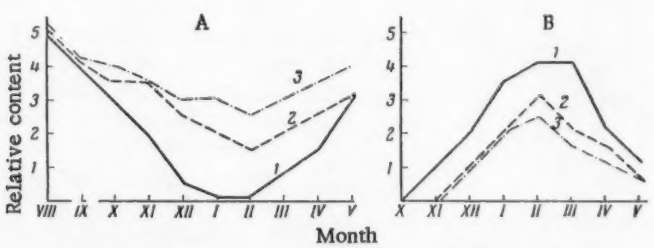

Fig. 2. Time course curves for starch (A) and fat (B) contents of one-year-old apple shoots. 1) Siberian berry apple; 2) variety Dolgoe; 3) variety Juicy white.

and "enforced" phases, as commonly determined by the time buds placed in favorable conditions begin to grow, does not reflect the complexity of processes taking place in the plant during this period. Published data [9-12] and our observations indicate that the time at which vegetative buds begin to grow (in this case, apple buds) is related to the specific nature of the plant's evolutionary development. Species which have originated in regions with a continental climate, where winter begins early and is free of prolonged thaws, have evolved the property of rapid transition through the period of preparation for resuming growth, since in winter they are not subjected to the danger of premature bud growth with ensuing death upon advent of frost conditions. Buds of southern species are incapable of growth over a long period and are thereby protected from death after winter thaws.

Nesterov [12] proposes the distinction between "duration of dormancy," as determined by the time required for buds placed in favorable conditions to grow out, and "depth of dormancy," as determined by the degree of protoplasmic shrinkage and the extent of conversion of reserve nutrients. "Depth of dormancy determines frost resistance, and duration of the dormant period determines winter hardiness of the plant" [12].

We believe that these two aspects of dormancy should be delimited, but we cannot agree with the identification of "duration of dormancy" as a criterion of winter hardiness, since the concept of "winter hardiness" includes the capacity to withstand all unfavorable winter conditions, and the author [12] refers only to mild climates, no determinations of winter hardiness in harsh continental climates having been made. "Depth of dormancy," as determined by protoplasmic shrinkage and degree of conversion of nutrient reserves, does in fact determine frost resistance.

Thus, during passage through the period of dormancy, two processes can be clearly distinguished in the plant—a process of preparation for growth in the following year, and a process concerned with the development of stability to low winter temperatures. The former is assessed according to the capacity of the plant to resume growth under favorable conditions; the latter according to protoplasmic shrinkage, to patterns of conversion of reserve nutrients, and to a whole series of other physiological changes. Evidently both processes can occur simultaneously in the plant. The duration of the former and the extent of the latter depend on hereditary properties of the species in question, i.e., on environmental conditions prevailing during the evolutionary formation of the species. For example, in Siberia greater frost resistance and winter hardiness of apple is correlated with shorter periods of dormancy and more pronounced tendencies to protoplasmic shrinkage, extensive hydrolysis of reserve nutrients, and reorganization of protoplasmic colloids.

SUMMARY

1. The most highly frost-resistant Siberian berry apple requires a short period for preparation of vegetative buds to resume growth (embryogenic process). Considerably more time is required for the fairly resistant varieties of Central Russia and the Michurin varieties.

2. The property of rapid passage through the embryogenic stage acquired by Siberian berry apple in the course of its evolution is transmitted hereditarily. Rennet varieties, which are closely related to Siberian berry apple, are also capable of completing this stage early.

3. In apple, vegetative buds do not emerge from dormancy simultaneously, but in sequence, beginning from the terminal bud, and the dormant period for the plant as a whole is therefore quite lengthy. It is, thus, impossible to judge whether the stage of "deep dormancy" has been completed according to the growth on a shoot of one or even several upper buds.

4. Evidently processes concerned with the preparation of vegetative buds to resume growth can take place not only at low, above-freezing temperatures, but also at sub-freezing temperatures.

5. During the dormant period, starch hydrolysis is more rapid and more extensive in the more highly frost resistant species and varieties than in the nonresistant varieties. On the other hand, fats and lipoidal substances are accumulated to a greater extent in tissues of resistant varieties during winter.

6. The swelling capacity of protoplasmic colloids of cortical parenchyma cells falls as frost resistance increases. It falls toward the middle of winter and rises toward spring.

7. Protoplasmic shrinkage in cortical parenchyma cells of one-year-old growth was observed throughout the dormant period in all the species and varieties examined. It is most pronounced in the middle of winter, and in resistant species.

8. In the study of physiological processes occurring during dormancy and their effect on frost resistance and winter hardiness it is necessary to take into account the fact that the balance between these processes may be different for species (varieties) which have evolved under different climatic conditions.

In conclusion, I wish to express my deep appreciation to the scientific director of this work, Professor P. A. Genkel'.

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THE EFFECT OF ANIONS ON COTTON UNDER CONDITIONS OF GRAVEL CULTURE

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As is well known, salting the soil has an osmotic and toxic action on plants. Increasing the content of water soluble salts in the soil causes an increased osmotic pressure of the soil solution which lowers the intensity of absorption of water and nutritive substances by the plant roots.

However, in a number of communications [1, 2, and others] it has been shown that at equal osmotic values the degree of action of different salts on plants is not the same, because of differences in their ionic state.

The problem of our work was to study the effect of various ions of sodium salts on plants. In order to lessen the osmotic action, we grew the plants with a good regime of water and nutrition.

The experiments were carried out in gravel culture by the method of Genkel' and Tsvetkova [3]. Raising plants by this process had an advantage for our purpose over soil or water culture in that it assured the best solubility of the salts, least buffer action of the solution, which is a property of soil culture, and permitted rapid change of the nutrient medium without damage to the plant itself. This also created good conditions for water supply and nutrition of the plants with good aeration of the roots and a strong mechanical support for the plants.

The experimental plants were Soviet cotton 1298 which were transplanted into the gravel as seedlings at the cotyledon leaflet stage. Then, when the plants had rooted, salts were added to the nutrient solution. Salting of the nutrient solution was carried out gradually by 0.05% and was added up to a 0.4% concentration of the anion in the form of Na_2SO_4, $NaHCO_3$, and NaCl. We used Knop's nutrient solution with addition of microelements.

The experiments were carried out over two years according to the same plan, with a difference in pH of the medium. In the 1958 experiments the pH was maintained by dilution with H_2SO_4 to pH 6.4, using the universal indicator. In the 1959 experiments the values of the pH were not equalized. After the appearance of chlorosis in the leaves, some of the plants were transferred to a solution of pH 6.4. At the end of the vegetative period all the plants were placed in a solution with pH 6.4.

The plan of the experiments by year was as follows:

1958 variant	1959 variant
Hellriegel	Knop
Hellriegel + Na_2SO_4	Knop + Na_2SO_4
Hellriegel + $NaHCO_3$	Knop + $NaHCO_3$
Hellriegel + NaCl	Knop + NaCl

The regularities in the growth and development of the plants in the two experimental years were the same When the sodium sulfate and sodium chloride were added to the solution, the plants developed normally, differing little from the controls. In the variant with addition of sodium bicarbonate there was a strong suppression of growth and development of the plants. In Figs. 1 and 2 we show the outward appearance of the plants in 1958 and 1959.

In the variant with Na_2SO_4 added to the nutrient medium in 1959 (Fig. 1) the cotton grew well and developed, but at the end of the vegetative period violet spots appeared on the leaves, the reason for the appearance of which was not established. There was also an obvious falling of leaves and fruit elements. In the experiments of 1958 using solutions with pH 6.4 this effect was not observed.

The data in Table 1 show a sharp increase in total water content in cotton leaves treated with NaCl, and also an increase in thickness of the leaf. This was noted earlier by us [4-6] in experiments with soil culture and chloride salting, but in the gravel culture the phenomena of succulency and moisture are shown more clearly, as can be seen in Fig. 3.

For an idea of the conditions of growth and development of the plants in a gravel culture we determined the osmotic pressure of the nutrient medium, the osmotic pressure of the cell sap of the leaves, and also the content of ash and chlorine in the cotton leaves. The osmotic pressures were determined by the cryoscopic method (Table 2).

As the data of Table 2 show, the reaction of the plants to salting is expressed as an increased osmotic pressure of the leaf cell sap. Here the specific action of the chloride ion appears in creating an increased osmotic pressure of the nutrient medium and in a great increase in osmotic pressure of the leaf cell sap. The

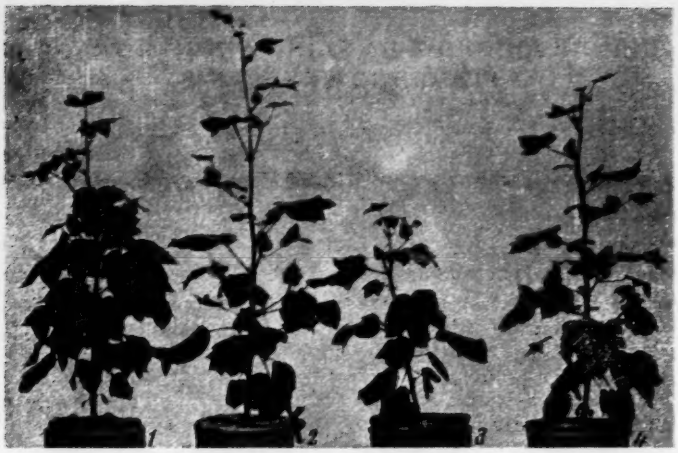

Fig. 1. Effect of anions on growth and development of cotton. Salts 0.4% as the anion; pH of solution 6.4. 1) Control; 2) Na_2SO_4; 3) $NaHCO_3$; 4) NaCl.

Fig. 2. Effect of anions and high pH of solution on growth and development of cotton. Salts 0.2% as the anion; pH not equalized. 1) Control; 2) Na_2SO_4; 3) $NaHCO_3$; 4) NaCl.

TABLE 1. Effect of Anions on Thickening of the Lamina and Water Content of Cotton Leaves under Gravel Culture Conditions (salt 0.4% as the anion)

Experimental variant	Leaf thickness, % of control	Water content of leaf	
		% wet weight	% of control
Control	100	77.9	100.0
Na_2SO_4	153.0	82.5	106.0
NaCl	128.0	188.0	90.8
$NaHCO_3$	104.0	105.0	81.2

creation of a high osmotic pressure in the leaf cell sap apparently occurs because of the great accumulation of the leaf ash elements and chloride. It is interesting that with sodium bicarbonate treatment the reaction of the plant to increased osmotic pressure of the nutritive medium is very weak, and the accumulation of ash elements by the cotton leaf is much reduced as compared to the other forms of salt addition.

The effect of specific ionic action is also shown in the changes in anatomical structure of cotton (leaf and stem).

In Table 3 we give data on the total area of leaf surface and the area of separate leaves (Aug. 18, fourth leaf; Oct. 27, eighth) and also of the structural elements of cotton leaves and stems.

The data of Table 3 indicate that the lowest leaf surface and lamina are found in plants whose solution was salted with $NaHCO_3$. Here the leaves are characterized by fine-celled covering tissue and also by a large number of cells and stoma per unit of leaf surface in both the lower and upper epidermis. This is especially observed on the leaves of the lower layers (fourth layer) to the end of the first half of the vegetative period. The structure of the leaves of the eighth layer differs in the much larger cells compared to the leaves of the same layer in cotton in the variant with Na_2SO_4, probably due to the transfer of the plant to the solution with pH 6.4 and the falling off of the lower leaves with chlorosis.

In comparison with the leaves of the control plants and especially with the plants in the variant with NaCl, the structure of the cotton leaves in the variant with bicarbonate shows a much more xeromorphic character in the covering tissues. Thickening of the lamina appears only at the end of the vegetative period and is very small, to 11% (cf. Fig. 3). Saidov [7] found a fine-

TABLE 2. Effects of Various Salts on Osmotic Pressure of Nutrient Solution and Leaf Cell Sap, and also on Content of Ash and Chloride in Cotton Leaves (salt 0.4% as the anion)

Experimental variant	Osmotic pressure, atm		% Content of air dried weight of leaves	
	nutritive medium	leaf cell sap	ash	chloride
Control	0.96	13.24	100	0.83
Na_2SO_4	3.62	19.84	131.2	0.61
NaCl	4.94	21.88	170.5	2.79
$NaHCO_3$	2.41	9.51	89.7	0.63

TABLE 3. Effect of Anions on Size and Anatomical Structure of Leaves and Stems of Cotton in a Gravel Culture (salt 0.4% as the anion)

Experimental variant	Area, cm^2		Leaf thickness, mm	No. in 1 mm of leaf				Diameter of stem, 1st internode mm	Thickness of tissue in stem radius, mm			Conducting vessels (in μ from-to)
	leaves on main stem	eighth leaf		upper epidermis		lower epidermis						
				cells	stoma	cells	stoma		pith	wood	bark	
Determined August 18, 1959												
Control	–	89.0	0.28	802.4	149.6	1032.0	309.1	–	–	–	–	–
Na_2SO_4	–	67.3	0.28	852.7	134.2	1178.5	322.5	–	–	–	–	–
NaCl	–	79.4	0.30	870.2	145.8	1055.7	278.6	–	–	–	–	–
$NaHCO_3$	–	28.8	0.25	1227.1	205.1	1466.0	451.5	–	–	–	–	–
Determined October 27, 1959												
Control	1073.4	111.4	0.23	975.0	162.5	1086.0	389.6	9.6	0.58	3.0	1.25	23-138
Na_2SO_4	775.0	76.1	0.34	1006.6	172.9	1377.0	366.4	6.4	0.86	1.48	0.86	14-92
NaCl	549.7	65.3	0.44	805.0	95.5	1042.3	250.3	6.4	0.52	1.61	1.08	14-83
$NaHCO_3$	129.6	57.6	0.26	998.5	121.3	1256.5	296.7	4.6	0.38	0.96	0.96	9-74

celled covering tissue and some leaf thickening when cotton was grown on soil salted with $MgCO_3$.

Cotton leaves grown on solutions with addition of NaCl had a rather high chlorine content (Table 2). However, no strong inhibition of leaf growth was found. The development of succulency was very marked. There was also a thickening of the leaf and large cells in the covering tissue at the end of the growth period when the leaves changed little in diameter.

Thus, the action of the chloride ion on the anatomical structure of leaves is shown by the increasing expansion of cells in the covering and mesophyllic tissues and weak differentiation of epidermal cells into stoma cells, as is confirmed by the data of Table 3 and Fig. 3. Black [8] grew Atriplex nastata with NaCl salting in a water culture and observed that the greatest leaf thickening developed with the least inhibition of growth of the lamina.

The action of sodium sulfate on the anatomic structures of the leaf is shown in the development of a xeromorphic leaf structure. This appears most strongly in the leaves of the eighth layer at the end of the growth period. Thus, a large area of the leaf assures a great number of cells and stomata per unit area of leaf as compared to the control plant. The thickening of the lamina under the influence of Na_2SO_4 develops quite markedly by the end of the vegetative period.

In gravel culture the conducting system of cotton is better developed in all variants of the experiment than in soil cultures [4]. In Fig. 3 we give a photomicrograph of a cross section of the wood with the conducting vessels and parenchymal cells in the first internode of the cotton stem. From the data of Table 3 and Fig. 3 it is evident that the development of the conducting system is more inhibited in cotton in the variant with sodium bicarbonate addition: smaller vessels, less stem diameter, and thickness of its tissues.

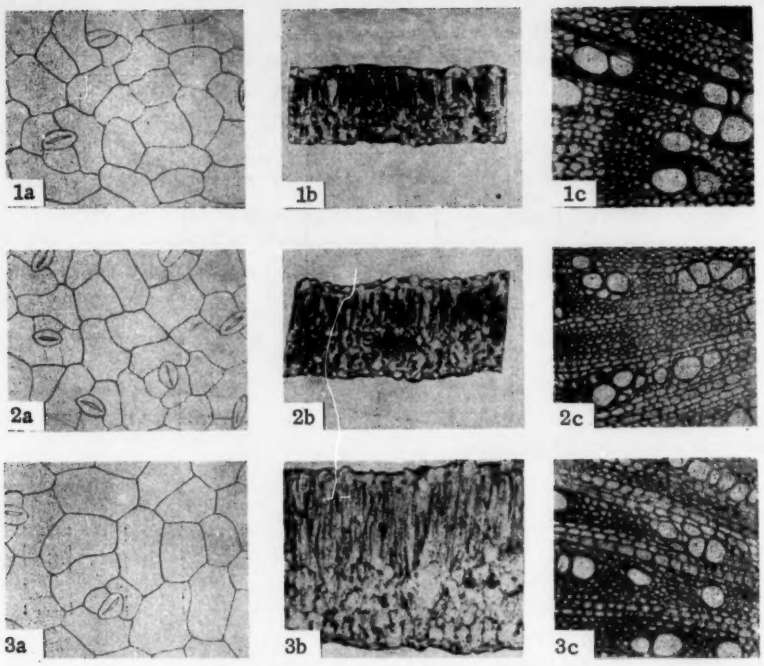

Fig. 3. Effect of anions on anatomical structure of cotton. Salts 0.4% as the anion; pH brought to 6.4 at end of experiment. 1) Control; 2) $NaHCO_3$; 3) NaCl; a) leaf epidermis (magn. 10 x 40); b) leaf thickness (magn. 80); c) cross section of woody stem of first internode (magn. x 154).

TABLE 4. Change in Content of Chlorophyll and Strength of its Bond with the Protein-Lipid Complex of Chloroplasts in Cotton Leaves when the Nutrient Medium Contains 0.2% HCO_3

Experimental variant	Position of leaf on stem	Total chlorophyll content of leaf, % of control	Extractability of chlorophyll, % of total content in leaf
Control	1st from below	100.0	21.64
$NaHCO_3$, pH ≥ 9	Same	64.3	39.84
Control	3rd from above	100.0	31.05
$NaHCO_3$, pH ≥ 9	Same	28.6	76.36

Salting with Na_2SO_4 has little effect on the anatomy of leaves and axial organs of cotton.

In studying the effect on cotton of salts with different anions we have paid considerable attention to the action of sodium bicarbonate. The use of gravel culture is very promising for this, as we said above. As is known, sodium bicarbonate has alkaline properties. An increase in the $NaHCO_3$ concentration in the nutrient solution is accompanied by a corresponding rise in the pH of the solution. Therefore, by growing the plant in a neutralized and unneutralized medium we could easily determine the action of the salt alone and also its action in combination with a rise in the pH of the solution.

There are a number of opinions as to the mechanism of action of carbonates on plants. However, most investigators tend to believe that under conditions of carbonate salting, plants suffer from a deficiency of iron, phosphorus, and calcium as a result of which growth stops and chlorosis follows in the leaves [9-14]. Rubin and Chernavina [15] consider that chlorosis in the leaves is connected with an insufficiency of iron for the plants and also a number of disturbances in the normal catalytic function of enzymes, the cytochrome oxidases. In view of the importance of this question, we decided to establish in more detail the conclusions from our experiments on salting with $NaHCO_3$. The plan of the ex-

periments for the year differed only in the change in pH of the nutrient solution.

In the experiments in which the solution was adjusted to pH 6.4 plant growth was suppressed. When the concentration of $NaHCO_3$ was brought to 0.2% (as HCO_3), scattered spots with fading of the chlorophyll appeared on the cotton leaves. These spots disappeared and then reappeared. However, the plants did not stop growing and at the end of the growth period had the form shown in Fig. 1.

In the experiments of 1959, salting of the nutrient solution was not accompanied by adjustment of pH. Thus, salting with $NaHCO_3$, besides increasing the content of HCO_3 in the solution, raised the pH of the medium.

When the concentration of HCO_3 was brought to 0.2% with a corresponding increase in pH to 9, a rapid decolorization of chlorophyll was found on the upper growing cotton leaves. The chlorosis occurred at the top of the plant, in the zone of most intense growth.

The chlorosis of the leaves was distributed along the stem from the top down and was accompanied by complete cessation of growth in the decolorized part of the plants. In the lower cotton leaves the green color remained stable. It was characteristic that in the leaves with chlorosis there was no wilting or loss of turgor. The leaves became brittle and turgescent with upraised edges; the leaf petioles were bent upwards elastically. Later, necrotic spots appeared on the leaves with chlorosis and at the growing points on the edges and between the veins of the leaves. After this they gradually died off. However, due to the activation of the axillary buds of the lower leaves the plant did not die. Fig. 2 shows plants which had Na_2SO_4, $NaHCO_3$, or NaCl added to the nutrient solution, during the period of marked chlorosis in the $NaHCO_3$–pH 9 treatment.

In connection with the occurrence of chlorosis of the leaves we carried out a determination of the total chlorophyll content in the lower green and the upper decolorized leaves treated with $NaHCO_3$. Also, by extracting chlorophyll with 60% alcohol [16] we determined the strength of the union of chlorophyll with the protein-lipid complex of the leaf chloroplasts.

According to the results in Table 4 we can conclude that the action of sodium bicarbonate and high pH of the medium causes chlorosis of the leaves, accompanied by a sharp decrease in total chlorophyll content in the leaf.

It is of interest that in the cotton leaf with chlorosis 76.4% of the total chlorophyll in the leaf is extracted by 60% alcohol, while in the control plants a total of 31.05% of the chlorophyll is extracted from leaves of the same layer. The easy extraction of the chlorophyll is evidence of weakening of its bond with the lipoprotein portion of the stroma of the leaf chloroplasts. Hence, we can express the opinion that the combined action of high pH and sodium bicarbonate leads to destruction of the bond in the complex chlorophyll-protein in leaf chloroplasts.

In our work of 1954 [5] this effect on cotton with chloride salting was less pronounced and was not accompanied by chlorosis of the leaves.

Thus, a concentration of sodium bicarbonate of 0.2% for the HCO_3 and a pH of 9 causes chlorosis of the leaves, decreased chlorophyll content, damage to the strength of its union with the protein-lipids of the stroma, and cessation of growth in the parts of the plant attacked. The action of $NaHCO_3$ is more marked in the young, vitally active parts of the plant. The lower leaves have a green color and their chlorophyll content is twice as great as in the upper young leaves with chlorosis. However, this effect is reversible. When one of the factors in the action of $NaHCO_3$, the pH of the medium, is removed, the above harmful effects are not found. The transfer of some of the plants in the variant with $NaHCO_3$ to the same concentration of sodium bicarbonate but with a pH in the nutritive medium of 6.4 leads in four to five days to a restoration of the green color in the upper, chlorotic leaves and a continuation of further growth and development of cotton.

SUMMARY

1. The varying effects on cotton of the anions of the salts Na_2SO_4, NaCl, and $NaHCO_3$ are shown most clearly in a gravel culture. In the order of decreasing harmful action on the plants, the salts can be arranged: $NaHCO_3$, NaCl, Na_2SO_4.

2. The limits of concentration for cotton in a gravel culture for $NaHCO_3$ are 0.2% calculated for HCO_3. At this concentration and a pH of 9, the growing parts of the plant develop chlorosis.

3. The occurrence of chlorosis in the leaves is accompanied by a sharp decrease in total chlorophyll content and weakening of its bond with the lipoprotein complex of the leaf chloroplasts. The reaction is reversible. When the nutritive medium is neutralized to pH 6.4 there is rapid restoration of the green color of the leaves and of the growth process.

4. The specific action on the plant of chloride ions in a gravel culture is shown by a rise in hydration of the leaf tissues, an increased osmotic pressure of the leaf cell sap and a great accumulation of ash elements and chlorine. This is probably due to the decreased osmotic pressure of the nutrient medium as compared to soil cultures.

5. The change in anatomical structure of the organs of cotton under the influence of different salts is not the same. The action of $NaHCO_3$ results in the development of xeromorphy of the cotton leaves and in strong inhibition of the development of the conducting system. The action of NaCl assures halomorphism in the structure of the organs with less variation from the normal in growth and development of the leaves and

the conducting system of the axillary organs. The structure of the organs of cotton when the nutritive solution is salted with Na_2SO_4 shows a little variation from the control plants toward increased xeromorphy of the leaf covering tissues.

In conclusion, I express thanks to Prof. P. A. Genkel' and Chief Scientific Worker B. P. Strogonov for consultation and advice in carrying out and reporting this work. I also thank Laboratory Assistants N. V. Balkin and L. Ya. Silkin for aid in the work.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. ***Some or all of this periodical literature may well be available in English translation.*** **A complete list of the cover-to-cover English translations appears at the back of this issue.**

SYNTHETIC ACTIVITY OF THE ROOT SYSTEM OF CORN DURING AMMONIA AND NITRATE NUTRITION

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Studies by D. N. Pryanishnikov and his school on nitrogen nutrition of plants serve as a basis for theory and practice in use of nitrogen fertilizers. Pryanishnikov [1] developed the problems of ammonia and nitrate assimilation by plants and the role of asparagine in ammonia nutrition. But in his studies the question was not investigated as to which organs accomplish processes of nitrate reduction to ammonia and the synthesis of amides.

D. A. Sabinin was the first to assume that the root system is not solely an organ of absorption and transportation of nutrient substances, but that multiform processes of organic substance synthesis occur in it [2]. Nitrogen in the form of mineral compounds coming from the soil is reprocessed in the root system and the larger portion of nitrogen enters the aboveground plant portion from the roots in the form of nitrogen compounds.

It is known from studies by A. L. Kursanov that sugars flowing from the leaves are oxidized in the root system to organic acids [3]. Hydroxy- and, especially, ketoacids, incorporate ammonia entering from the soil or formed up on nitrate reduction and form different organic nitrogen compounds which are subsequently "exported" to other organs. It is established at present that the root system synthesizes, depending on conditions of plant nutrition, over 25 different forms of nitrogen compounds. This complex cycle of A. L. Kursanov opens new aspects in the activity of the plants' root system, and their study is of great importance.

TABLE 1. Secretion of Corn Sap on Different Sources of Nitrogen Nutrition and at Different Stages of Growth and Plant Development (in ml per plant for the first 2 hours of collection)

Nitrogen source	7-8 leaves	Panicle flowering	Beginning of milky ripeness
Ammonia	1.3	4.8	6.0
Nitrate	1.4	5.3	6.3

In our study we investigated the problem of the synthetic activity of the corn's root system in relation to ammonia and nitrate sources of nutrition. We used the sap method developed by D. A. Sabinin.

The experiments were conducted in aqueous cultures in vessels of 6 liters with Krasnodarskii hybrid 1/49 corn variety. Plants were cultivated on a nutrient mixture with NH_4NO_3 as nitrogen source. Well developed plants were chosen for the experiment.

Six days before commencing the experiment the corn was transferred to a nitrogen-modified complete Knop mixture. A portion of the plants was transferred to vessels with a nitrate source of nitrogen, another portion to one with ammonia nitrogen. The state of plants is characterized by the following data.

The average weight of the plants' green mass was: on the ammonia source at the 7-8 leaf stage—58.8 g; panicle flowering—266.2 g; the beginning of milky ripeness—533.2 g; on the nitrate source—respectively, 60.2, 258.5, 520.0 g.

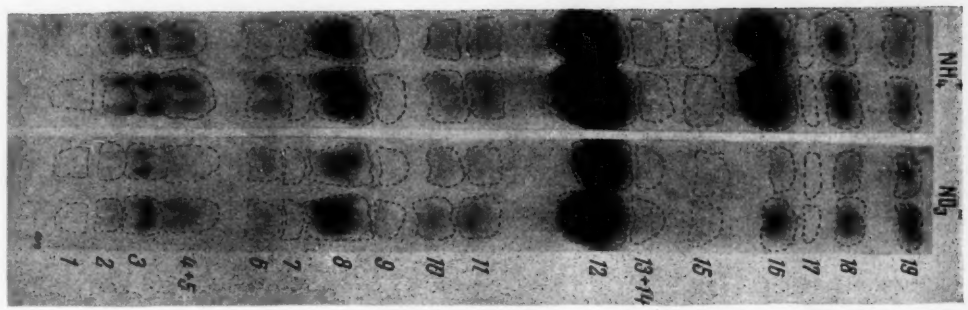
The aboveground portion was cut and the sap collected into sterilized receptacles. The sap was collected at the following stages: 7-8 leaves, panicle flowering and the beginning of milky ripeness. The sap was collected for the first two hours after cutting the plant. The well developed plants secreted sap abundantly (Table 1).

Determinations were made on the sap of total nitrogen, nitrates with disulfophenolic acid, ammonia with Nessler's reagent and measurement on a photoelectrocolorimeter, and amino acids by paper chromatography. Amino acids after partition were developed by 0.5% ninhydrin in acetone. In order to obtain uniform coloration and subsequent quantitative determination the chromatograms were treated by copper nitrate in acetone. Amino acids from the colored spots were extracted with 75% ethyl alcohol and density of coloration was measured on a photoelectrocolorimeter with a blue filter. Their concentration was calculated by calibration curves established for each amino acid. For total nitrogen the sap on the ammonia background was digested with sulfuric, and from the nitrate back-

TABLE 2. Secretion of Nitrogen with Corn Sap from Different Sources of Nitrogen Nutrition at Different Stages of Growth and Plant Development (for the first 2 hours of collection, calculated per 100 plants)

Nitrogen source	7-8 leaves					Panicle flowering					Beginning of milky ripeness				
	total nitrogen		organic nitrogen		mineral nitrogen, mg	total nitrogen		organic nitrogen		mineral nitrogen, mg	total nitrogen		organic nitrogen		mineral nitrogen, mg
	mg	%	mg	% of total nitrogen		mg	%	mg	% of total nitrogen		mg	%	mg	% of total nitrogen	
Ammonia	67.70	100	63.10	90.5	4.60	261.60	100	238.10	91.0	23.50	268.8	100	225.00	83.7	43.8
Nitrate	44.24	63.5	35.83	81.0	8.41	189.84	75.4	150.50	79.3	39.34	205.38	76.3	164.31	80.0	41.07

Note: A determination of total and mineral nitrogen was made, while the organic nitrogen was calculated by difference.



Amino acid content in sap in relation to the nitrogen sources (7-8 leaf stage). Solvent: a mixture of butyl alcohol, acetic acid and water in proportions of 4 : 1 : 1. Amino acid identification was conducted by markers and color reactions with isatin. 0.08 ml of fresh sap was applied to each spot. Amino acids: 1) Cystine; 2, 3) lysine plus histidine; 4, 5) asparagine plus arginine; 6) glutamine; 7) aspartic acid; 8) serine; 9) glycine; 10) glutamic acid; 11) threonine; 12) alanine; 13, 14) proline and tyrosine; 15) tryptophane; 16) γ-aminobutyric acid; 17) methionine; 18) valine; 19) leucine.

TABLE 3. Amino Acid Content in Corn Sap on Different Sources of Nitrogen Nutrition, at Various Stages of Growth and Plant Development (in mg calculated per 100 ml of sap)

	7-8 leaves		Panicle flowering		Beginning of milky ripeness	
Amino acid	ammonia	nitrate	ammonia	nitrate	ammonia	nitrate
Cystine + cysteine	7.00	4.50	7.50	6.62	5.30	4.25
Lysine + histidine	14.07	10.56	14.22	10.57	30.10	12.50
Asparagine + arginine	7.43	4.97	23.20	8.80	33.40	21.90
Glutamine	3.30	2.63	2.45	2.08	14.90	14.90
Aspartic acid	9.08	5.06	6.54	4.63	12.05	9.90
Serine	25.00	18.76	35.00	24.20	53.50	36.30
Glycine	4.27	2.30	6.40	3.88	4.84	4.50
Glutamic acid	6.15	4.04	5.82	4.54	14.60	12.90
Threonine	6.00	6.14	9.96	9.25	20.00	11.90
Alanine	107.85	75.00	110.00	81.20	61.40	46.30
Proline	Present	Present	Present	Present	Present	Present
Tyrosine	6.66	3.39	9.40	5.00	Traces	Traces
Tryptophane	7.20	Traces	Traces	Traces	Traces	Traces
γ-aminobutyric acid	18.20	5.00	8.86	5.90	4.66	3.62
Methionine	1.20	Traces	Traces	Traces	3.56	3.50
Valine	9.97	7.74	9.25	8.80	12.60	7.25
Leucine	4.05	3.19	5.62	5.20	6.92	5.25
Total amino acids	237.43	149.41	254.22	180.49	277.83	194.97
Total amino acids, %	100	62.8	100	71.0	100	70.2

TABLE 4. Beta-hydroxyamino Acid Content in Corn Sap on Different Sources of Nitrogen Nutrition at Various Stages of Growth and Plant Development (in mg calculated per 100 ml of sap)

Nitrogen sources	7-8 leaves	Panicle flowering	Beginning of milky ripeness
Ammonia	31.00	45.96	74.50
Nitrate	24.90	33.45	48.20

TABLE 5. Content of Diamino Acids and Amides in Corn Sap on Different Sources of Nitrogen Nutrition at Various Stages of Growth and Plant Development (in mg per 100 ml of sap)

Nitrogen sources	7-8 leaves	Panicle flowering	Beginning of milky ripeness
Ammonia	24.80	39.87	78.40
Nitrate	18.16	21.45	49.3

ground with phenolsulfuric acid, and the nitrogen was determined by a micro-Kjeldahl method.

According to data by D. N. Pryanishnikov the ammonia nitrogen enters plants faster than the nitrate nitrogen [1]. According to our data (Table 2), it can also be seen that secretion of nitrogen with the sap on an ammonia nitrogen source is higher than on a nitrate source. This indicates a retarded utilization by plants of the nitrate nitrogen in processes of synthesis. The nitrates required a preliminary reduction to ammonia, and only subsequently can they become incorporated into organic acids.

An intensified formation of organic nitrogen compounds and their secretion with the sap occurs in the root system when the source is ammonia nitrogen. But when the source is nitrate nitrogen, the quantity of organic nitrogen is smaller during all stages of plant development. The quantity of mineral nitrogen from either source is quite insignificant, and does not exceed 20%. We found the following amino acids in the fresh sap: cystine, histidine, lysine, asparagine, arginine, glutamine, aspartic acid, serine, glycine, glutamic acid, threonine, alanine, proline, tyrosine, tryptophane, γ-aminobutyric acid, methionine, valine, and leucine. Basically all the essential amino acids (arginine, lysine, histidine, threonine, leucine, valine, tryptophane, methionine), transferred to the aerial portion of the plant, are synthesized in the root system.

The number of amino acids was similar in the cases of ammonia and nitrate sources, but the quantity of above-mentioned amino acids in all growth stages was larger when the nitrogen source was ammonia (Table 3).

In corn sap the predominant amino acid was alanine, the concentration of which at the 7-8 leaf stage was as high as 45.5-50% of the total amino acids. At the milky ripeness stage the quantity of alanine was about half of this—22.1 to 23.7% of total amino acids. The second place quantitatively belonged to serine. The quantity of serine at the 7-8 leaf stage was 10.5 to 12.5%; at the stage of beginning of milky ripeness it increased to 18.7-19.3% of the total amino acids. An exceptionally marked difference was manifested by γ-aminobutyric acid at the stage of seven-eight leaves: when the nitrogen source was ammonia the γ-aminobutyric acid concentration was 18.2 mg, while when the source was nitrate, it was only 5.0 mg per 100 mg of sap, i.e., less than 1/3 the former.

As the plant progresses in growth the concentration of β-hydroxyaminoacids (that of serine and threonine) increases in the sap (Table 4).

Simultaneously the number of nitrogen-saturated compounds (diamino acids and amides) increases in the sap. The content of these is considerably larger when the nitrogen source is ammonia than when it is nitrate (Table 5).

While at the 7-8 leaf stage the concentration of diamino acids was 10 to 12.1%, at the beginning of milky ripeness stage it was 25.3% to 28.2% of the total amino acids. The data indicate that at the stage of milky ripeness there is apparently a lack of carbohydrates in the root system and amino acids abundantly rich in nitrogen form.

SUMMARY

The transformation of nitrate and ammonia nitrogen in the root system of corn was studied by the phloem sap method in water cultures during the 7-8 leaf stage, panicle flowering stage and commencement of the milk stage.

The general amount of nitrogen supplied, nitrogen of mineral compounds and amino acids in the sap was taken into account. The amount of nitrogen supplied depends on the form of the nitrogen fertilizer. More nitrogen as a whole and in the form of organic compounds is supplied during ammonia nutrition than during nitrate nutrition.

The following amino acids were detected in the sap: cystine, histidine, lysine, arginine, aspartic acid, serine, glycine, glutamic acid, threonine, alanine, proline, tyrosine, tryptophane, α-aminobutyric acid, methionine, valine, and leucine. The amides asparagine and glutamine were also detected. Alanine predominates in corn sap. Up to 45-50% of the total amount of amino acids consists of alanine during the 7-8 leaf state, whereas during the milky stage the alanine content is half as great and comprises only 22-24% of the total amino acid content.

The same types of amino acids are encountered when the two forms of nitrogen are supplied, but greater amounts are present during ammonia nutrition.

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ON THE DORMANCY OF PLANTS UNDER CONDITIONS OF IRRIGATION WITH SALT SOLUTIONS

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The reaction of plants to such adverse environmental conditions as low temperature, drought and abnormal soil salinity is manifested outwardly by a retardation of their growth processes.

The growth rate of perennial plants is retarded or completely arrested at certain times of the year, when such plants become dormant. According to Genkel' and Oknina [1], the assumption of the dormant condition in winter is characterized by changes in the composition of protoplasm in perennial plants, which results in a separation of the cytoplasm from the cell wall and a consequent interruption of intercellular connections. This coincides with cessation of growth.

The assumption of the dormant state by plants also takes place at temperatures unfavorably high for their growth, as has been demonstrated by Vegis [2, 3] for Hydrocharis.

Plants which grow in arid places or in regions where the soil has a high salinity show a periodicity in their growth during the vegetative period. Changes in the water content of the soil or in its salt concentration may check the growth of such plants.

The most marked periodicity in the growth of plants occurs in soils irrigated with salt solutions. Under these conditions, it is not unusual to find that plant growth is retarded during the periods of irrigation, to be resumed at a higher rate after irrigation has ceased. This depression of the growth rate during irrigation with salt solutions cannot be explained merely in terms of "starvation" of the plant—as was at one time believed to be the case—for the amounts of nitrogenous material and carbohydrate per unit dry weight of the plant organs noticeably increases during irrigation [4, 5]. Thus, the impression is created that under conditions of irrigation by salt solutions plants pass into a state of short-term dormancy and that this is reflected in a diminution in intensity of growth.

With a view to testing this particular hypothesis we carried out investigations into the condition of the protoplasm in plants growing under conditions of salt solution irrigation. For these experiments, which took place at the K. A. Timiryazev Institute of Plant Physiology, USSR Academy of Sciences, Moscow, the following plants were used: cotton, sunflower, barley, corn and tomatoes. The condition of the protoplasm in the leaves and shoots was studied using living sections and sections fixed in Helly's fluid and submitted to the usual cytological methods, or the particular method described by Genkel' and Oknina [6].

Experiment 1. For this experiment, tomato plants of the variety "Earliana" were used. The plants were supplied by V. A. Solov'ev and were grown in 6-kg plant pots containing a good loamy soil. The seed had been sown and germination completed by June 10 and by June 16 the plants were producing good shoot growth. Before sowing the tomato seed, the dry soil was irrigated with a solution of NaCl, and this irrigation was repeated on June 24 after the appearance of the seedling shoots above soil level. This irrigation resulted in a concentration equivalent to 0.1% Cl per absolute dry weight of soil. During the period of growth, soil moisture was maintained at a level of 70% saturation.

The salinization of the soil had an inhibitory effect on the growth rate and shoot formation of the plants. According to V. A. Solov'ev, the control plants had attained a height of 25 cm by July 7, whereas the experimental plants did not exceed 12 cm. When the fruit was gathered, the control plants were found to yield 380 g per bush, whereas the experimental plants only yielded 32 g. These facts indicate quite clearly the inhibiting effect which irrigation with salt solution has on the growth processes of plants.

For the examination of protoplasm in experimental plants, the third leaf from the top was taken. Results of such an examination made on July 30 are illustrated in Fig. 1. It is evident from this figure that the protoplasm of plants grown in salinized soil is strongly contracted away from the cell wall.

Experiment 2. For this experiment the author used barley plants of the variety Wiener. These were grown by E. F. Ivanitska in 6-kg plant pots containing a good loamy soil. The soil moisture was maintained at 70% of full saturation. Seed was sown on May 14 and the germinating shoots began to show on May 18. The irrigation fluids used in this experiment consisted of specially prepared salt solutions of equivalent amounts

TABLE 1. Growth and Development of Barley Plants Irrigated by Two Different Salt Solutions

Experimental variant	Height of plants (cm)	Dry wt. of whole plants (g)
Control	57.5	2.83
Sulfate irrigation	55.1	0.96
Chloride irrigation	47.5	0.60

of either chloride or sulfate ions [4, 7]. Irrigation of the soil was carried out on three different occasions, using in each case solutions equivalent to 0.2% salts per absolute dry weight of soil. These irrigations took place before sowing, on May 20 and on June 8; thus, the ultimate salt concentration reached 0.6%.

As in the previous experiment performed by the author, irrigation of the soil had an inhibitory effect on the growth and development of the plants but the inhibitory action of the same amounts of the two different ions was very unequal. The observations of E. F. Ivanitska [Table 1] showed that the growth and development of the barley plants was inhibited much more strongly by chloride salinization than by sulfate.

Plant stems were used in order to examine the condition of the protoplasm in the tissue of the barley. The results of such an examination on June 23 are illustrated in the photomicrographs (Fig. 2).

Analysis of Fig. 2 indicates that under conditions of irrigation with salt solutions the protoplasm of the plant cells undergoes important changes. In the case of plants treated with sulfate ions, the protoplasm shows a relatively slight contraction away from the cell wall and the plasmodesma are retained, whereas in the case of those treated with chloride ions the protoplasm is strongly contracted and the plasmodesma have disappeared.

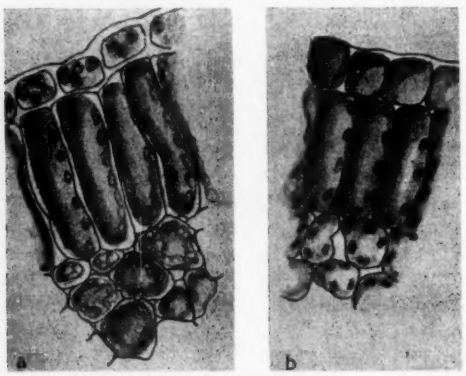

Fig. 1. Condition of the protoplasm in the leaves of tomato plants grown in salinized (a) and non-salinized (b) soil (× 400).

In addition to the above observations, it is important to note the changes which take place in the interstices between the cells of plants undergoing chloride salinization. If the anatomical sections of organs from sulfate and chloride salinized plants (Fig. 2) are compared, it will be seen that chloride salinization results in a diminution in the areas of cells with an accompanying sharp increase in the size of the cell interstices (Fig. 2b). It is possible that in the cell interstices there may be an accumulation of highly hydrophilic substances, particularly pectins, on which the water exchanges of plants in salinized soils depend [4]. If this is so, then these hydrophilic substances may be responsible for the changes in the condition of the cell protoplasm associated with the assumption of the dormant condition.

The facts described above suggest that when plants are irrigated with solutions of various salts the effect of the salt on the plant is to bring about changes in the condition and properties of the protoplasm. This results in the disruption of normal connections between the cells and a shrinkage of their protoplasm away from the cell walls. The physiological effect of these cytoplasmic changes is an interference with the normal growth processes such that the externally apparent growth and development of the plant is retarded.

However, as was pointed out above, irrigation of the soil with salt solutions may sometimes result in an intensification of growth among treated plants instead of retardation. This occurs particularly when plants which have been irrigated initially with salt solution are exposed to a much lower salinity at a later date.

In order to substantiate the theory which we have developed regarding the relationship between the contraction of the protoplasm away from the cell wall and the retardation of growth, it is important to establish a close connection between the change in the condition of the protoplasm and clearly defined periodicity in the growth process. In order to demonstrate this connection

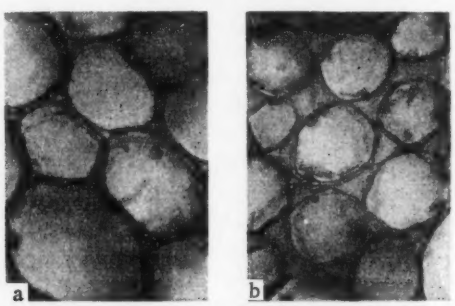

Fig. 2. Condition of the protoplasm in barley stem cells growing under conditions of sulfate solution (a) and chloride solution (b) irrigation (× 400).

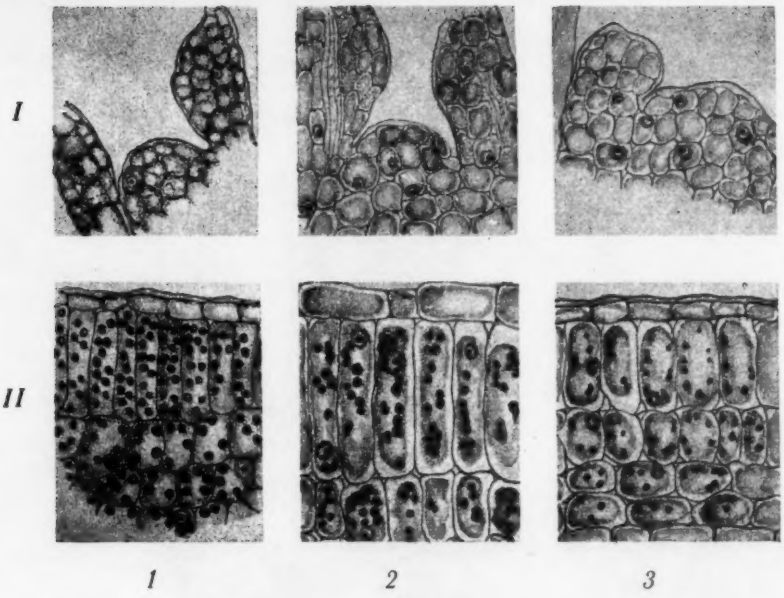

Fig. 3. Condition of the protoplasm at the growing points and in the leaves of cotton plants growing in salinized nutrient solution. 1) Control; 2) treated with Na_2SO_4; 3) treated with NaCl (x 400). I) Growing point; II) leaf.

we carried out a special experiment, using plants grown by L. P. Lapina.

Experiment 3. This investigation was carried out using cotton plants of the variety 1298. The plants were raised in gravel culture according to the technique of Genkel' and Tsvetkova [8]. The technique required two vessels—a larger outer vessel and a smaller inner vessel (a glazed bowl with an opening in the bottom). The inner vessel was filled with gravel and placed in the outer vessel which had previously been filled two-thirds full of nutrient solution, prepared according to the formula of Chesnokov and Bazyrina [9]. Throughout the course of the experiment the initial volume of nutrient solution was maintained by adding the appropriate amount of tap water to the vessel every day. The nutrient solution in the vessel was changed once a month. Such a method enables plants to be raised under the most favorable conditions for rapid growth and allows the experimenter to regulate the salt regime of the nutrient mixture without disturbing or damaging the root system.

Seeds were sown in the gravel on May 30 and the young shoots first made their appearance on June 5. Salinization of the nutrient medium was carried out gradually, using sodium sulfate and sodium chloride in isotonic concentrations. The first salinization of the medium was carried out on June 8, the last on June 26, by which date the concentration of sodium chloride had reached 0.9% and that of sodium sulfate 1.44%.

Observation of the plants on July 31 showed that salinization of the nutrient medium had produced a sharp reduction in their growth rate. When the chloride and sulfate were supplied in isotonic concentrations, it was the chloride which exhibited the greater toxicity to plant growth and development. The toxicity was manifested both in the effect on growth in height of the cotton plants and also on the formation of leaves. Sodium chloride-treated plants showed an increase in the number of leaves as compared with controls, but their leaf area was greatly reduced.

The third leaf from the apex of the main stem was used for examining the condition of the protoplasm in the cotton plants. In addition, the growing point was sectioned and examined. The results of examinations carried out on July 31 are illustrated in Fig. 3. Observation of this figure reveals that salinization of the nutrient medium resulted in a strong contraction of the protoplasm from the cell walls and the disappearance of plasmodesma. By contrast, the cells of cotton plants grown in the absence of added salts show no such contraction of their protoplasm and the presence of plasmodesma testifies to the retention of intercellular connections. Judging from the wide incidence of protoplasmic contraction, the effect of the salts added to the nutrient solution is manifested throughout the whole plant body, including the apical growing points.

We regarded it as very important to establish that the change in condition took place at the time of the

TABLE 2. Effect of Salinization of Nutrient Medium on the Growth and Development of Cotton Plants

Conditions of cultivation and date of sampling	Experimental variant	Height of plant		No. of leaves	No. of buds	No. of flowers	No. of bolls
		in cm	as % of control				
Before removal of salts from medium (July 31) (control)	without salinization	30.9	100	13.5	4.5	–	–
	Na_2SO_4	18.5	100	7.5	2.5	–	–
	NaCl	17.4	100	15.5	2.0	–	–
After removal of salts from medium (Sept. 18)	without salinization	47.7	154.3	26.5	6.0	2.0	2.5
	Na_2SO_4	31.6	170.8	25.5	5.0	–	1.0
	NaCl	35.8	205.7	38.0	5.0	1.0	2.0

Fig. 4. Growth and development of cotton plants in salinized nutrient media. Plants which had been grown under conditions of sodium chloride treatment (1), sodium sulfate (2), and without salinization—control (3).

plants' transition from retarded to intensive growth. Hence, we felt it necessary to produce experimentally the periodicity of growth which is often found in plants growing naturally in soils of varying salinity. In order to carry this out, on July 31 we transferred all the cotton plants from the salinized nutrient media to other non-salinized media.

Subsequent observations showed that the growth intensity of the plants, which had previously been retarded by salinization, increased in every case. On September 18 a report on the same plants was submitted, and this indicated that their growth intensity was considerably higher than that of the controls which had never been subjected to salinization (Table 2).

It is a matter of interest that the cotton plants which had suffered the greatest depression of growth, i.e., those growing in nutrient media with added sodium chloride, manifested a more intensive growth and development after removal of the salt than did the sodium sulfate-treated plants. At the end of the experiment, the former group of plants had grown taller and produced more fruiting elements than had the latter and closely approximated in size to the controls (c.f. Table 2 and Fig. 4).

By means of this experiment we have established the reason for the greater depth of dormancy produced in plants as a result of sodium chloride salinization as compared with sodium sulfate, namely that the former salt has a more inhibitory action on the growth processes and mobility of nutrient substances than has the latter. When the sodium chloride is removed from the vicinity of the roots, the previously unusable nutrient substances are energetically mobilized and serve to supply the growth processes with the raw materials they require.

We have already demonstrated that under conditions of salinization the leaves not only perform their normal photosynthetic function, but also serve as secretory organs (for the elimination of excess salts) and as storage organs. Under conditions which favor rapid growth of the plant, the materials previously stored in the leaves are rapidly made available for the formation of new plant organs [5].

Observations of the condition of the protoplasm in these plants was carried out by the author on September 22. The third leaf from the top of the plant was used for the examination and the results are illustrated in Fig. 5.

An analysis of Fig. 5 indicates that in the control plants and in the experimental plants which were initially treated with sodium sulfate there is no contraction of the protoplasm away from the cell walls and

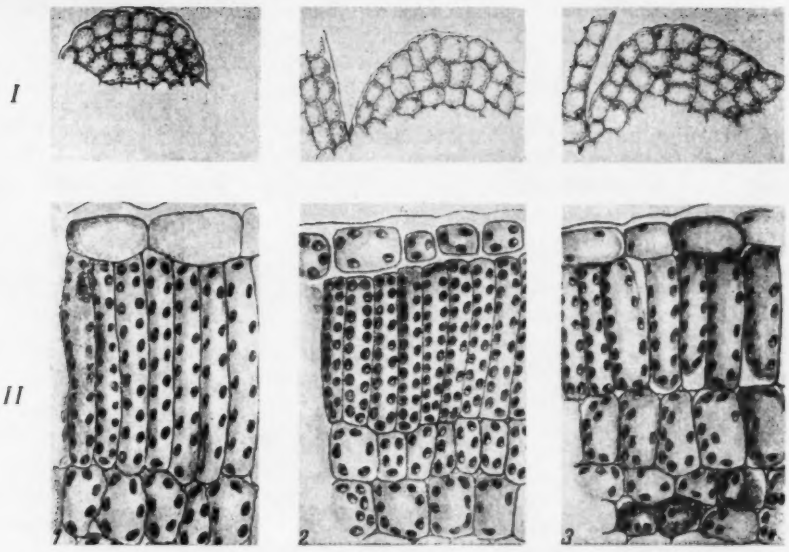

Fig. 5. Condition of the protoplasm at the growing points and in the leaves of cotton plants in salinized nutrient media. 1) Control; 2) after treatment with Na_2SO_4; 3) after treatment with NaCl (× 400). I) Growing point; II) leaf.

that plasmodesma are faintly discernible. Plants which were initially grown in nutrient solution containing sodium chloride, however, show some degree of residual contraction of the protoplasm in certain of their leaf cells, but no contraction in cells of the growing points.

From these results it may reasonably be inferred that plants growing under conditions of abnormal salinity suffer a contraction of their protoplasm away from the cell wall, a change which results in the severing of intercellular connections. Washing out the excess salt from the substrate restores the intercellular connections. The effects of salinization are therefore reversible, although the reversibility of the process is conditioned to a large extent by the toxicity or otherwise of the ion concerned. Thus, in the case of the plants used in the previous experiment, removal of sodium chloride from the medium did not restore all the cells to their normal condition; there was a certain amount of residual contraction occasioned by the toxicity of the chloride ion. In plants grown under the conditions of high sodium sulfate concentration and then transferred to a normal nutrient medium there was no residual effect of the salt. It should also be noted that the degree of initial protoplasmic contraction induced by the chloride was considerably greater than that induced by the sulfate solution, at least in the majority of cases. Moreover, the number of cells with contracted protoplasts was directly proportional to the degree of growth suppression in the whole plant and to the depth of dormancy induced by salinization.

It is the basis of our hypothesis that contraction of the protoplasts destroys the intercellular connections in many parts of the plant and thus brings about a diminution in the exchange of water and nutrients between cells. A reduction in the concentration of water and nutrient materials, particularly at the growing point, causes retardation of growth in the plant as a whole. During this phase, nutrient materials, e.g., carbohydrates and proteins, tend to accumulate and be deposited in the various organs of the plant body where they are not available for growth. In our opinion, this immobilization of food materials is one of the principal causes of the depression of growth of plants growing in solutions of high salinity.

The phenomenon of contracted protoplasts is of wide occurrence in nature. Not only does it occur normally during winter dormancy, but also during the summer, if nutrient materials are lacking, or during drought. Under these conditions plants cease to grow and pass into a dormant state. The state of dormancy is also observable in seeds undergoing maturation [10, 11], in the tubers of rubber plants and potatoes [12, 13], in the buds of shrubs and trees [14-17], and in the growing points and leaf rudiments of grasses and perennial herbs [1, 18-21].

It should be noted here that Tulaikov [22], in 1912, held the opinion that the deleterious effect of salts was due to their capacity to plasmolyze protoplasts, thus causing the latter to contract away from the cell walls with a consequent loss of turgor in the organs of the plant. Such a point of view is clearly in disagreement with the actual facts. However, N. M. Tulaikov was unaware of the phenomenon whereby protoplasts of adjacent cells could cease to be in communication with

one another and was thus led to his erroneous conclusions.

SUMMARY

1. Plants growing under conditions of high salinity exhibit a change in the condition of their protoplasts, which become contracted away from the cell walls. This phenomenon leads to a rupture of the plasmodesma and a cessation of intercellular transport. These changes in the protoplasm of cells result in a disturbance of the normal growth processes of the whole plant such that development is arrested.

2. Contraction of the protoplasts is a reversible process. When plants which have been growing in a substrate of high salinity experience more favorable conditions (e. g. by washing the salt away from the substrate), the normal state of the protoplast is resumed, intercellular connections are restored and there is a rapid increase in the intensity of growth.

3. This process of protoplasmic contraction is to be regarded as a protective adaptation, which enables the plant to pass into a state of dormancy and thus resist the unfavorable factors of its environment.

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THE SYNTHESIS OF SOME THIOUREA DERIVATIVES AND THE DETERMINATION OF THEIR ACTIVITY AS GROWTH SUBSTANCES

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pp. 86-91, January-February, 1961

One of the factors in biological activity of plant growth stimulators is their chemical nature. On the basis of data in the literature on this question, a ring system which in most cases is the radical of an aromatic hydrocarbon is of utmost importance for growth activity [1]. If this is a benzene ring and has substituents in positions 2 and 4, then usually the compound which contains this radical has growth-promoting activity [2]. An exception in this respect is, for example, 2,4-dinitrophenoxyacetic acid, which is inactive for plants [1].

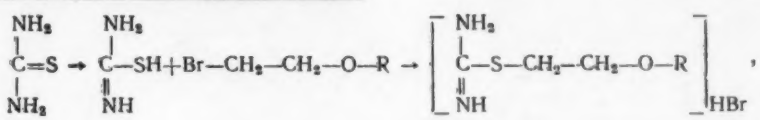
The nature of the side chain is of great importance to growth activity. In particular, the activity of homologs of 2,4-dichlorophenoxyacetic acid (2,4-D) which have a phenyl radical on the carbon furthest from the carboxyl is considerably lower than for 2,4-D. Acids of this series with an even number of carbon atoms in the side chain have growth-promoting activity, while acids with an odd number of carbon atoms are inactive [3,4].

Growth activity is also found in such compounds as 2,4-dichlorophenylmercaptoacetic acid [5] and sodium 2,4-dichlorophenoxyethyl sulfate [6]. The first of these compounds contains a sulfur atom instead of an ester oxygen, and the second a sulfuric acid residue instead of a carboxyl.

Thus, there is no doubt that growth-promoting activity is often shown in a number of compounds which are analogs of 2,4-D; therefore, we felt that we should carry on the search for new growth stimulators in this direction. Thiourea also shows action on plants and can be used as an agent for disturbing the resting state of potato tubers [7].

In view of the above considerations, we have synthesized three compounds which are S-derivatives of thiourea and some 1-bromo-2-phenoxyethanes. The reaction for the formation of this type of compound is based on the ability of thiourea to react in the tautomeric form of the sulfur atom with substances which contain a mobile halogen [8]. The products formed in this reaction are hydrobromides of phenoxyethane isothioureas.

The reaction occurs according to the scheme:



where R is the phenyl, 4-chlorophenyl, or 2,4-dichlorophenyl radical.

EXPERIMENTAL METHOD

To obtain the hydrobromides of the isothiourea derivatives we used as the reacting substances thiourea (pure) and some bromophenoxyethanes: 1-bromo-2-phenoxyethane; 1-bromo-2-(4-chlorophenoxy)ethane; and 1-bromo-2-(2,4-dichlorophenoxy)ethane. The physical constants of these bromophenoxyethanes were close to the constants given in the literature [9,10,11].

To carry out the reaction we placed equimolecular amounts of thiourea and the corresponding bromophenoxyethane in a conical flask and heated the mixture in alcohol solution on a water bath, under reflux. The thiourea dissolved and reaction occurred, ending after 25-30 minutes. After the reaction had ended, heating on the water bath was continued for a further 30 minutes and the mixture was left for 10-12 hours for crystallization of the reaction product. The crystalline product was separated from the mother liquor on a Büchner funnel and washed with small amounts of pure alcohol, then with ethyl acetate, and was then dried.

We thus obtained: 1) phenoxyethane thiourea bromide with a yield of 79%; 2) 4-chlorophenoxyethane thiourea bromide with a yield of 70%; 3) 2,4-dichlorophenoxyethane thiourea bromide with a yield of 82% of the theoretical.

The phenoxyethane thiourea bromide was a colorless crystalline substance with a melting point of 149°, soluble in water and alcohol, poorly soluble in acetic

TABLE 1. Growth Activity of Thiourea Salts of Phenoxyethanes (in %)

Name		Activity at concentration, mg/liter		
full	abbreviation	20	10	5
2,4-Dichlorophenoxyacetic acid	2,4-DA	100	100	100
Phenoxyethane thiourea	PET	261	265	268
4-Chlorophenoxyethane thiourea	4-PET	131	134	203
2,4-Dichlorophenoxyethane thiourea	2,4-PET	140	177	180

TABLE 2. Effect of Phenoxyethane Thiourea Hydrobromides on Root Formation in Bean Seedlings

Concentration, mg/liter	Eight day seedlings		Eight day seedlings	
	av. no. of roots per seedling	length of portion on which roots are found, mm	av. no. of roots per seedling	length of portion on which roots are found, mm
	Indole acetic acid		Indole acetic acid (potassium salt)	
100	94	81	57	53
50	103	81	49	47
10	75	81	23	41
5	63	70	15	29
2	62	60	12	29
	2,4-Dichlorophenoxyethane thiourea bromide (2,4-PET)		2,4-Dichloroethane thiourea	
100	62	61	40	51
50	59	72	35	44
10	56	70	20	34
5	47	71	12	29
2	50	63	20	37
	4-Chlorophenoxyethane thiourea bromide (4-PET)		4-Chloroethane thiourea	
100	71	84	9	56
50	90	80	44	49
10	52	71	19	37
5	63	78	14	31
2	33	62	10	12
	Phenoxyethane thiourea bromide (PET)		Phenoxyethane thiourea	
100	60	71	14	12
50	35	66	14	25
10	53	62	14	21
5	43	58	7	9
2	31	46	10	16
			Water (control)	
			13	23

acid, and insoluble in ether and benzene. The free base melted at 128°.

For the formula $C_9H_{13}N_2OBrS$:

	Found, %	Calculated, %
Nitrogen	9.90	10.01
Bromine	29.02	29.22
Sulfur	11.30	11.59

Molecular weight: found, 278.56; calculated 276.

4-Chlorophenoxyethane thiourea bromide was a colorless, crystalline substance with a melting point of 127°. Soluble in water and alcohol, insoluble in benzene. The free base melted at 113°.

For the formula $C_9H_{12}N_2OClBrS$:

	Found, %	Calculated, %
Nitrogen	8.68	8.98
Chlorine + bromine	37.03	37.09
Bromine	25.60	25.65
Sulfur	10.34	10.27

Molecular weight: found 311.04; calculated 311.64.

2,4-Dichlorophenoxyethane thiourea bromide was a

colorless, crystalline substance with a melting point of 173°. Poorly soluble in water, easily so in alcohol, insoluble in benzene.

For the formula $C_9H_{11}N_2OCl_2BrS$:

	Found, %	Calculated, %
Nitrogen	8.00	8.11
Chlorine + bromine	43.60	43.72
Bromine	23.06	23.16
Sulfur	9.26	9.27

Molecular weight: found 343.30; calculated 346.00.

In the titration of alcoholic solutions of these compounds with aqueous 0.1 N alkali we isolated the free bases in the form of glittering scales which because of partial decomposition had a particularly unpleasant odor. The base which contained the 2,4-dichlorophenol residue was the most unstable and under the influence of aqueous alkali was converted into a thick sticky mass.

The growth activity of these thiourea salts was determined by the action of their aqueous solutions with different concentrations on the length of the primary root in germinating corn seeds. The activity of 2,4-D was taken as 100% [12].

The results of the determinations are given in Table 1.

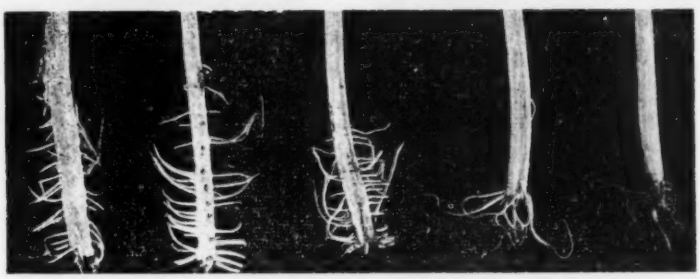
As Table 1 shows, these isothiourea compounds show very high growth activity compared to 2,4-D, with the PET preparation showing growth of the primary root only, and the 4-PET and 2,4-PET preparations showing an effect not only on the primary (main) root, but also on the additional roots, and in this case, with the primary root in a number of cases showing a number of branches.

This growth activity was studied in several sets of experiments on bean seedlings with the Turetskaya method [13, 14]. The results of this series of experiments are shown in Table 2.

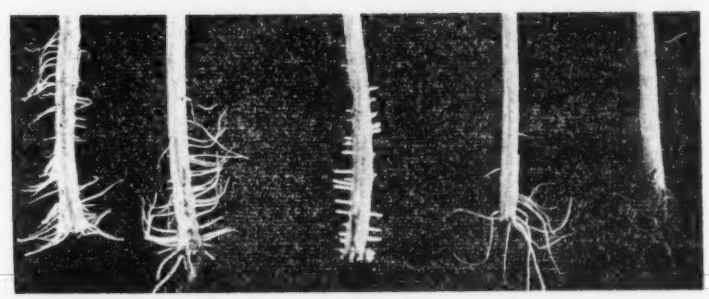
In one set of experiments (repeated three times) we used eight-day bean seedlings immersed 90 mm in water. During the rooting period the temperature in the morning was 21°, in the day, 23°, and in the evening, 22°. The results were taken on the eighth day and are given in Table 2.

Table 2 shows that the phenoxyethane thiourea bromides have a high enough activity as compared to the potassium salt of indoleacetic acid. If the activity of the indoleacetic acid is taken as 100%, then at concentrations of 50 mg/liter preparation 2,4-PET has an activity of 57.2%, and preparations 4-PET and PET have 87.3 and 34.9%, respectively. At concentrations of 10 mg/liter the preparations show activities of 70.6, 69.3, and 78.3%, respectively. Thus, preparations of 2,4-PET and 4-PET can be considered active with respect to root formation, while preparation PET is evidently slightly active.

In another set of experiments (repeated five times) we used 10-day seedlings grown at a temperature of 20-23° on the average. The results of this set of experiments are also shown in Table 2.



A



B

Effect of phenoxyethane thiourea bromides at different concentrations on root formation in bean seedlings. A) 50 mg/liter; B) 100 mg/liter. Formation from left to right by: indole acetic acid; 2,4-PET; 4-PET; PET; water (control).

The results of the second series of experiments are similar to those of the first set. After we had calculated the results of the second series of experiments, we selected one of the most characteristic seedlings from the group of seedlings treated with solutions of these preparations at concentrations of 100 mg/liter and 50 mg/liter and photographed them (figure, A and B).

The figure shows that preparations of 2,4-PET and 4-PET can form more vigorous roots, but in smaller quantities than can indole acetic acid.

In one set of experiments whose results are not given here, there appeared on the seedlings treated with solutions of 2,4-PET and 4-PET at concentrations of 100 mg/liter and 50 mg/liter a thickening and rupture of the tissues along the whole length which was immersed in water. The temperature during the rooting period of the seedlings varied from 26 to 32°. In our opinion, such temperatures favor a toxic action of these preparations. Almost all the seedlings at these concentrations failed to show one root.

SUMMARY

1. We have synthesized three derivatives of isothiourea which are new and have not previously been described in the literature.

2. We have studied the effect of these compounds on the length of the primary root in sprouting corn seeds. The phenoxyethane thiourea bromides are more active in their effect on the length of the primary roots of sprouting corn seeds than is 2,4-D.

3. We have determined the effect of phenoxyethane thiourea bromides on root formation in bean seedlings by the method of R. Kh. Turetskaya. We have showed that these compounds are close to indoleacetic acid in their activity on root formation.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.

ON THE ROLE OF PHYSIOLOGICALLY ACTIVE SUBSTANCES OF THE PHYTOHORMONE AND VITAMIN TYPES IN HETEROSIS PHENOMENA IN CORN*

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Since the time of Darwin, who established the harmfulness of self-pollination and the usefulness of cross-fertilization in plants, many efforts have been made to explain the nature of this phenomenon. It is particularly sharply expressed in the lowering of productivity in self-pollinated lines, and as a sharp increase in productivity in heterosis hybrids.

Fairly recently a summary by Sprague [1] was published, in which the literature on the genetic nature of heterosis is reviewed in rather great detail from the point of view of Morgan genetics, while very few papers are cited on the physiology and biochemistry of this phenomenon [2, 3, 4, and others]. Insofar as the role in heterosis of physiologically active substances of the phytohormone type is concerned, Sprague mentions only the work of Robbins [5], and Whaley and Long [6], who have shown that heterosis hybrids are able to synthesize more growth than their self-fertilized parental forms. However, this statement is contradicted by the data of a well known geneticist-biochemist, Avery [7], who found that the amount of free auxins in the hybrid seeds is not always greater than that of their parents. The important part physiologically active substances of the phytohormone type play in the heterosis phenomenon is indicated in the book by Soding [8], where, however, it is not very specific. In the Laboratory of Plant Physiology and Biochemistry in the Ukranian Institute of Plant Breeding, Selection, and Genetics it was decided to gather as much information as possible on the aspects of physiology and biochemistry of heterosis by means of independent investigations, using corn as an example. We were particularly interested in studying the role of physiologically active substances in the appearance of hybrid vigor when crossing self-pollinated lines.

It is known that the hybrid plants which show heterosis have a greater growth energy, as a result of which they accumulate more organic mass in the same period of time than the self-pollinated parental plants and thus become comparatively much larger in size. It is known also that the larger plant size may result from one of three causes: larger cell size, larger number of cells, or both of these. It has been considered generally that the cell size is determined mainly by the conditions of the second growth phase – the phase of cell elongation. This process is affected by a number of factors of the external and internal environment, among them the presence in the meristems of physiologically active substances, mainly auxins. Insofar as the number of cells is concerned, that is determined by the nature of the first embryonic phase of growth, i.e., it depends on the rate of cell division and on the duration of the active state of meristematic tissues, the capacity of the constituent cells to stay for a long time in the state of embryonic growth. The duration of this phase and rate of cell division in turn depends on the supply of water, food, and physiologically active substances to the meristem. Among the latter the most effective are usually considered to be the growth substances of the yeast extract group.

Of course, physiological processes taking place in the plant organism, meristems, and other parts depend also on factors of the environment (temperature, light, etc.), but these factors do not concern us now, since they affect hybrids in the same way as their parental lines. The greatest interest from our point of view is the study of the capacity of hybrids and their parents to synthesize and use physiologically active substances which have a direct and specific action in the growth processes: growth substances of group A – auxins, Kholodnyi's blastanin, heteroauxins, and group B – biotin (vitamin H), thiamin (vitamin B_1), riboflavin (vitamin B_2), pyridoxine (vitamin B_6), pantothenic acid (vitamin B_3), folic acid (vitamin B_C), nicotinic acid (vitamin PP), etc.

In 1956 and 1957, we determined the content of growth substances of Group A in a number of corn hybrids (about 20), using Kholodnyi's method of extraction [9], and the Avena coleoptile bio-assay technique. We stated that in most cases this indicator in the hybrid occupied an intermediate position to that of parental forms, as

*Report at the Conference on the Heterosis Theory in Plants and Animals, held by Hungarian Academy of Sciences, September 5-9, 1960, Budapest.

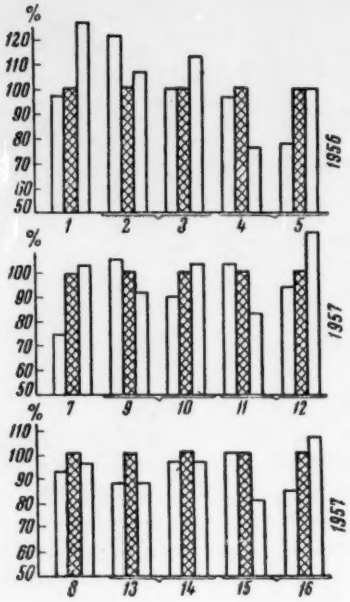

Fig. 1. The relative content of growth substances of group A in heterosis hybrids and parental lines of corn. The content of growth substances in hybrids is taken as 100% (in the graph, the shaded column in each triad). On the left the value of maternal parent, on the right, that of paternal parent, is shown. The triad pairs 2 and 3, 4 and 5, 9 and 10, 11 and 12, 13 and 14, 15 and 16 illustrate the content of growth substances in hybrids with direct and reverse crossing.

can be seen in Fig. 1. From this we concluded that the larger size of heterosis hybrids is caused not by the larger size of the constituent cells, but by their greater number. This a priori conclusion was then supported by the direct determination of size and number of cells in hybrid and their self-pollinated parents (see table).

It is interesting to note that the American geneticist G. Shull [10], who has done much work on clarification of the mechanism of hybrid vigor, and who had proposed the name "heterosis" itself for explanation of this phenomenon, considered one of the effects of heterosis to be intensive cell division. Higher meristematic activity of heterosis hybrids was also pointed out by Burkholder and McVeigh [11], as well as Paddick [12].

It became evident to us that attention should be shifted to growth substances of group B, which have an important part in the process of cell division. It is known that yeast fungi are particularly sensitive to this group of physiologically active substances; because of this, this group is occasionally called a "yeast complex." The presence of such substances in the substrate, such as a plant tissue extract, is determined by means of a bio-assay with yeasts [13, 14]. The addition of a small amount of extract from the tested plant tissue, containing the components of "yeast complex," to the yeast growing in the nutrient medium causes intensive budding and growth of yeast. Growth is quantitatively determined turbidimetrically with a nephelometer.

Using this yeast bio-assay technique we determined the content of growth substances of group B in tissues of several dozen heterosis hybrids, as well as their parental lines, and established the fact that in the great majority of cases the content of "yeast complex" in seeds, seedlings, and leaves of corn hybrids is considerably higher than in the corresponding parts of their inbred parents. Some results of these determinations are given in Fig. 2, where daily increases in yeast growth are given in percent, the 100% value being the increased growth of yeast suspension to which the extract from hybrid leaves was added (shown by shaded column in the graph).

Having established these facts, we have attempted to understand the theory involved, and were able to formulate the following: physiologically active plant substances do not act on the plant organisms separately, but as a part of complicated, well balanced, and, as a rule, well coordinated, systems. However, they are not always present in optimal concentration. In such cases, the viability of the organism is decreased and, sometimes even death of the organism takes place.

One may suppose that one of the most important reasons for weak growth, generally injured state, and low productivity of self-pollinated lines, specifically of corn, is the incompleteness and lack of balance in the systems of physiologically active substances. To these we should refer not only growth substances, but also enzymes, particularly of the oxidation-reduction group. The successful crossings of two such "suboptimal" parental lines may lead to "attaining the optimum" and to the "balancing" of the active systems which were present in the organism of inbred parents. As a result of this, the zygote and hybrid organism developing from it form a more complete system of physiologically active substances, able to insure a better rate of metabolism, i.e., an organism with high metabolic activity, with high viability, and, therefore, with high productivity. But, it also may happen in reverse: if in the crossed lines the systems of physiologically active substances are defective, incomplete, and similarly suboptimal, i.e., if they lack the same components, then as a result of combination of their systems during fertilization no such "attaining of the optimum" takes place. On the contrary, previously existing inbalance in parents may appear again or even become more intensive, leading to the injury and even death of the young hybrid organism. Is this not the explanation of the occasionally observed lethality of the hybrid seedlings? In other words, the favorable results

Anatomical and Morphological Characteristics of Leaves of Several Corn Hybrids and their Parental Forms

Type of corn	Area of leaves		Area of epidermal cells		Number of cells in 1 cm²	
	cm²	%	μ²	%	total number	%
1956 Experiment						
SP-VIR 26*	525	87.4	3939	113.1	25 390	88.4
Hybrid Iskra	601	100.1	3483	100.0	28 710	100.0
SP-VIR 27	512	85.3	4232	121.5	23 632	82.3
1957 Experiment						
SP-VIR 26	324	83.7	2217	103.7	43 945	96.1
Hybrid Iskra	387	100.0	2138	100.0	45 703	100.0
SP-VIR 27	334	86.3	2270	106.2	43 750	95.1
SP-VIR 3	330	92.9	1772	83.9	46 875	100.8
Hybrid VIR 3 × VIR 4	366	100.0	2112	100.0	46 484	100.0
SP-VIR 4	302	82.5	2136	101.2	41 503	89.3
SP-VIR 4	302	93.8	2136	121.2	41 503	92.6
Hybrid VIR 4 × VIR 7	322	100.0	1762	100.0	44 824	100.0
SP-VIR 7	307	95.4	2587	146.8	36 718	81.9

*SP—Self-pollinating.

of the crossing of self-pollinated lines (heterosis) may be obtained only in the case where the crossed lines are of different physiological quality, and, because of this, supplement each other during crossing.

Here we must make certain improvements in precision. When we speak about possibility of more perfect systems of physiologically active substances arising in the zygote as a result of the union of parental gametes with incomplete and unbalanced systems, it is impossible to believe that this process is merely an act of mechanical mixture of some chemical combinations of their groups. In the parental gametes, more probably, there are no ready active systems. There are only "original" specific chemical structures, predetermining such direction of metabolic processes in the zygote and the organism arising from it, which leads, or, more exactly, may lead, to the synthesis of a definite physiologically active system. Such "original" chemical structures, specific for each parental line, present in the parental gametes, enter into chemical interaction during fertilization and may undergo such changes that the product of the action — new original chemical structures — may turn out to be the bearer not of the sum of parental qualities, but of an entirely new quality or qualities. This is indicated by completely different results of direct and reverse crossings. One should not forget that the formation of the qualitatively new original chemical structure during fertilization may take place under strong influence of external environmental conditions. Further, in the interrelated and reciprocally conditioned flow of the consequent chemical reactions of the intercellular metabolism, the original direction of such reactions may be basically changed under the influence of external and internal environments. This may lead to the completely unexpected results.

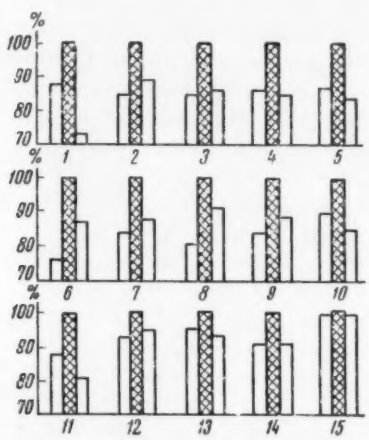

Fig. 2. The relative content of growth substances, Group B, in the leaves of corn hybrids and their parental lines, according to the yeast bio-assay. Hybrid values are taken to be 100%. In each triad the left column refers to the maternal parent, the right column to the paternal, and the middle one to the hybrid.

In the light of ideas expressed, our hypotheses concerning "attaining the optimum" and "balancing" of physiologically active systems seem simple and mechanical. However, we will consider it useful to use them temporarily in our further work, with those correc-

tions concerning the mechanism of transfer of "hereditary information" which we have just made.

Our explanation of the heterosis effect by "attaining the optimum" of the suboptimal physiologically active systems in parents during their crossing found confirmation recently in the investigation of Bulgarian scientists Kudrev, Stoilov, and Georgiev [15]. In their work, the reprint of which the authors were kind enough to send us in the spring of 1960, they investigated the nature of the reaction of isolated roots taken from the seedlings of several varieties of corn to the addition to the nutrient medium of various vitamins – B_1, B_2, B_6, PP. They have shown that "the varieties of corn which react in the same way to the addition of vitamins to the nutrient medium [i.e., according to our conceptions professing qualitatively similar systems of physiologically active substances – the authors] during hybridization do not give a high heterosis effect."

The logical continuation of our study, described above, should be in two directions:

1) By sorting out those complicated systems of physiologically active substances which were mentioned above, as far as possible specifying their qualitative and quantitative compositions in heterosis and nonheterosis hybrids, and their parental lines. On doing so, our working hypothesis may be either confirmed or disproved.

2) By developing a method for determination of physiological differences in self-pollinated (inbred) lines of corn in order to help plant breeders in selecting parental pairs resulting in hybrids showing heterosis.

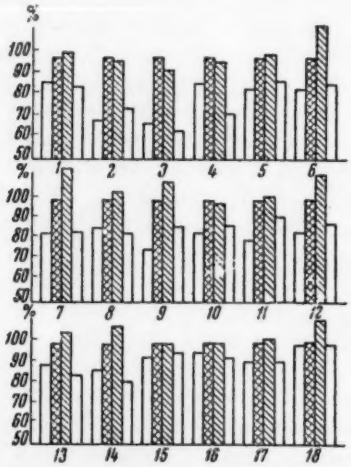

Fig. 3. Daily growth substances of yeast in the experiment. In each tetrad the first column stands for the growth increase of yeast when extract from the female parent is added to the nutrient solution; the fourth – from the male parent; the second – from tissues of the hybrid; and the third – mixture of extracts from tissues of two parents.

The work in both of these directions was started simultaneously, but was carried out at a different rate.

In our studies on determination of the component of physiologically active systems in hybrids and their parents, we at once met with methodological difficulties. The search for rapid chemical and physiological methods of separation, identification, and quantitative determination of the "yeast complex" components were not successful when chromatography, electrophoresis, and polarography [16-22] were used, because of the very low concentration of physiologically active substances in plant tissues. We had to use biological methods, using for the bio-assay cultures of specific microorganisms [23-25], but only preliminary steps have been taken in this direction.

The work in the second direction, i.e., developing a method of determination of the physiological differences in self-pollinated lines of corn in order to make a prognosis of the crossing results, was carried out on a larger scale and gave results which could be used for the practical selection work.

We deliberately tried the simplest, truly empirical, but sufficiently rapid, biological method.

The method consisted of the following [26]: the water extract from leaves of self-pollinated lines was prepared with hot digestion; the ratio of dry weight to water was 1:50. These extracts were tested for growth substances of group B using baker's yeast (Saccharomyces cerevisiae) for the bio-assay. The yeast was weighed, 500 mg per liter added to Gaiduk's nutrient medium, and this was poured into the test tubes (20 ml each), to which then were added the extracts from seeds, or, preferably, from the young leaves of corn prepared earlier (0.5 ml per test tube). Then (and this is the essential part of the method) to the same test tube is added 0.5 ml of the mixture of extracts from the tissues of the two inbred lines which are being compared (0.25 ml of one line extract plus 0.25 ml of the other line extract). All test tubes are then placed in a thermostatic chamber (temperature 28-30°) for 24 hours. The growth of the yeast during a 24-hour period, determined turbidimetrically in a nephelometer, served as a measure of the quantity and quality of physiologically active substances in the tested extracts and consequently in those lines of corn from which the extracts were obtained. A priori we considered that if a physiological difference exists between two inbred lines of corn, the addition of the extract mixture to the yeast sample should give a greater growth of yeast than the addition of the various amounts of pure extracts.

To check the validity of this hypothesis we initially included in the experiment only those self-pollinated (inbred) lines, which on crossing gave heterosis hybrids, i.e., those which are physiologically different (in our terminology). We added to the yeast solution not only extracts from the parental line tissues and mixtures of such extracts, but extracts from the hybrid tissues, too.

Partial results of these experiments are given in Fig. 3, where daily increases of yeast are given in percent ; the 100% value represents the growth increase obtained by addition of the young hybrid leaf extract to the yeast extract (shaded column).

In considering Fig. 3, two facts become obvious: 1) leaf extracts of the great majority of hybrids showed a high positive effect on the yeast tested, as compared with their inbred parental forms; 2) the extract mixtures from the leaves of parental lines showed almost the same positive effect on the bio-assay as extracts from the leaves of the corresponding hybrids, and, in a number of cases, even higher.

Here we may make the following conclusions: 1) in self-pollinated lines of corn, physiologically active substances of group B (yeast complex) are less perfected, suboptimal and less balanced than in heterosis hybrids obtained by their crossing; 2) during hybridization, in case of the successful selection of parental pairs, certain "attaining of the optimum" takes place in physiologically active systems, as a result of which they become more perfected; 3) the effect of "attaining of the optimum" of the physiologically active systems may be obtained by mechanical mixture of extracts from the tissues of self-pollinated lines; however, theoretically, it may happen only in those cases when the lines are physiologically different; 4) consequently, the positive effect obtained by the mixture of extracts from the self-pollinated line tissues to the yeast assay might indicate their physiological difference.

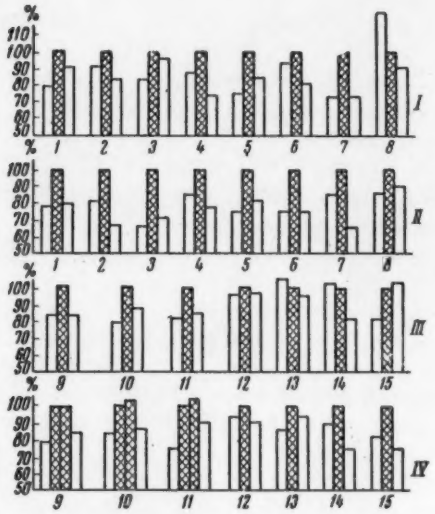

Fig. 4. Relative reactions of yeast growth to the addition to the nutrient medium of extracts from the tissues of parental plants — separately, and in mixtures (rows I and III) and relative plant height (rows II and IV). In the first case, as 100%, the yeast growth increase resulting from the addition to the nutrient medium of extract mixture (middle column), and in the second case (rows II and IV) as 100%, the height of the hybrid is taken (middle column). In tetrads 9, 10, and 11, the second and third columns stand for the height of hybrids from direct and reverse crossing. The numbers under triads (and tetrads) stand for the numbers of parental pairs used in the experiment.

There is nothing surprising in the fact that the mixture of extracts in a number of cases produced a higher positive effect than the extracts from the hybrid tissues: the hereditary properties of parents need not always appear in their progeny; often completely different results are obtained from reciprocal crossing of the same parental pair.

Nevertheless, we consider that as a result of these investigations a practical possibility is opened to determine the presence or absence of physiological differences in self-pollinated lines, with respect to the nature of their physiologically active systems, and with some degree of probability to foretell the results of a cross between inbred lines of corn, as to the possibility of obtaining hybrids with a high growth rate, i.e., which show heterosis.

This rather bold conclusion which we make necessitated checking. We attempted to carry out such a check in 1960.

Up through the spring of 1959, we tested the physiological differences of approximately 20 parental pairs for a number of self-pollinated corn lines which were sent to us from different selective stations of the USSR. The seeds were planted and crossing was carried out between selected parental pairs, among which some, according to our hypothesis, should have given progeny showing heterosis (according to the growth rate), and the others should not.

In the spring of 1960 the obtained hybrid seeds were planted in order to check the correctness of the prognosis which we had made. At the present time we have results only of the measurements of plant height and area of the most developed leaves. Part of this data is given in Fig. 4.

From the material given it can be seen that the prognosis of crossings which we made were satisfactory, insofar as increase of growth rate in hybrids is concerned, in those cases where plants showing physiological difference were crossed. Thus, when the addition to the yeast solution of the extract mixture from the leaves of parental form gave a positive effect, the height of the hybrids, as well as the area of their leaves (not shown in the drawing) generally turned out to be considerably greater. However, in a number of cases (numbers 8, 13, 14, 15), particularly in those places where the reaction to the addition of parental tissues to the nutrient mixture was not clear, certain increase of these values (the height of stems, the area of leaves) in hybrids, as compared to parents, still takes place. This once more indicates that the reaction of the yeast test to the addition of the extract mixture from the tissues of parental line to the nutrient me-

dium, showing the presence or absence of physiological difference, should be considered only as an additional indicator of behavior of crosses from the self-pollinated lines of corn.

SUMMARY

1. In the phenomenon of heterosis in plants, at least in corns, a large role is played by the physiologically active substances of the type of phytohormones and vitamins. This is indicated by a high meristematic activity in hybrids showing heterosis, resulting in a larger size of their organs, mainly because of the larger number of cells.

2. According to the content of growth substances of group A (determined by means of the Avena coleoptile test), the heterosis hybrids of corn generally are not superior to their self-pollinated (inbred) parents, but occupy intermediate positions between them. In contrast, the content of the growth substances of group B (determined by means of a yeast bio-assay test) in heterosis hybrids is higher than in their parental lines in the majority of cases.

3. One may suppose that one of the reasons for the weakened state of the self-pollinated lines of plants is a suboptimal condition (incomplete and unbalanced) of their physiologically active systems. One of the reasons for heterosis is the appearance of hybrids, during crossing of two physiologically different inbred lines, of the capacity for synthesizing more complete and balanced systems of the physiologically active substances as a result of successful combinations of the metabolic types of parental lines.

4. The effect of "attaining the optimum" and "balancing" of physiologically active systems may be obtained by means of mechanical mixture of extracts from the tissues of inbred parents. This is indicated by the positive reaction of the yeast assay to the addition of the extract mixture from the tissues of those inbred lines which give on crossing progeny showing heterosis. The positive reaction of the bio-assay is absent, if to the yeast extract is added a mixture of extracts obtained from the tissues of physiologically similar inbred lines which on crossing do not give heterosis. Here we have a possibility of judging concerning the presence or absence of physiological difference, based on the reaction of the yeast assay to the addition of the extract mixture from the tissues of the tested inbred lines. Consequently, we may guess to some degree the behavior of progeny resulting from their crossing.

5. An experimental check on the application of this method for prediction of the crossing results gave satisfactory results in 1960. It showed that the reaction of the yeast test to addition of extract mixtures from tissues of parents may be a supplementary indicator of the behavior in crosses of self-pollinated lines of corn. However, the further development of the method and a wider testing of it are still needed. Equally, it is necessary to develop further the theoretical side of this question.

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EFFECT OF DERIVATIVES OF NUCLEIC ACID METABOLISM ON THE GROWTH AND FLOWERING OF Perilla nankinensis

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INTRODUCTION

The study of the physiological nature of flowering processes in plants has always been connected with clarification of the role of both external factors regulating this process and internal factors through which the onset of flowering is regulated. Because of this, it is natural that the most important facts in this direction have been obtained by studying photoperiodism and plant responses to temperature changes, quality and intensity of light, and other important factors of the environment, and, on the other hand, clarification of changes in the metabolism taking place in the leaves and apical meristems, and the part played in this process by the substances of high physiological activity – auxins, synthetic growth substances and vitamins [1-5].

Recent investigations have shown that the flowering of many long-day varieties, among them seedlings of winter forms and biannuals, is promoted by gibberellins – recently discovered, highly active substances. It has been made clear that gibberellins do not affect the flowering of short-day varieties [6-10]. In connection with this a hypothesis has been proposed that the flowering of short-day plants is affected by a group of substances of another type, which are nitrogeneous in nature, and connected with the increase in nitrogen concentration and protein metabolism (Chailakhyan [11]).

In recent years the attention of many investigators has been devoted to the derivatives of nucleic acids, since some of these turn out to be substances greatly influencing growth and morphogenic processes. The most active metabolites are purine and pyrimidine bases, one of the analogs of which – kinetin (6-furfuryladenine), isolated in 1955 in Skoog's laboratory [12] from deoxyribonucleic acid of animal origin – was distinguished by particularly high and variable physiological activity [13, 14].

The role of the compounds related to nucleic acids in growth processes turns out to be highly significant and at the present time altogether obvious. The changes in the amount and content of nucleic acid in cells are intimately connected with physiological activity of various zones of such axial organs as the root [15-18]. These changes take place in different ways at various stages of morphogenesis under various conditions of nutrition and illumination [19-21], and play an essential role in the appearance of various types of tumorous growth in plants [22-24]. It has also been established that kinetin and other 6-substituted thio- and amino-purines stimulate germination of the seeds which react favorably to light, replacing the action of red light [25, 26]. Purines and pyrimidines induce the process of morphogenesis of apical buds in cultures of isolated undifferentiated tissues [27, 28].

Considerably less clear is the role of compounds related to nucleic acids during change of plants from vegetative growth to reproductive development. However, in this direction, too, there are analytical data as well as direct results obtained in the experiments studying the effect of metabolites and antimetabolites of nucleic metabolism on plant flowering. Thus, determinations of Turkova and Zhdanova [29], as well as Butenko [30], have shown that the nucleic acid content in both long-day and short-day plants is increased when they begin to flower. The action of antimetabolites of nucleic acid metabolism in all experiments leads to the retardation of flowering, e.g., when the short-day plant Xanthium (Xanthium pennsylvanicum) is acted upon by 5-thiouracil in conditions of short-day (Salisbury and Bonner [31]); when plants of another short-day species – one-leafed Streptocarpus wendlandii – are acted upon by thiouracil and a decrease of ribonucleic acid and protein is observed (Hess [32]); when beans (Vicia faba) are affected by thiouracil in long-day conditions (Evans, [33]).

Opposite results – the acceleration of plant flowering – were obtained in all experiments in which derivatives of nucleic acid metabolism were used. Spraying plants of red-leafed Perilla (Perilla ocymoides, var. nankinensis) with weak kinetin solution led to flowering when plants received incomplete ten-day induction by short days (Lona [34]). Spraying of some fruit trees with xanthine and uracil led to their flowering, and in the leaves of flowering plants the increase of ribonucleic acid to deoxyribonucleic acid was observed (Kessler, Bak, and Cohen [35]). The acceleration of flowering was also ob-

tained when 1) henbane plants (Hyoscyamus niger) were treated with hydrolyzate of ribonucleic acid (Sarkar [36]); 2) beans (Vicia faba) were treated with a mixture of nucleosides of ribonucleic acid (Evans [33]); 3) peas (Pisum sativum) were treated with guanosin (Highkin [37]); 4) meadowgrass (Poa annua) was treated with a mixture of the nucleic protein fraction of diffusate from vernalized winter rye plants and natural auxins from the same plants (Tomita and Tohoku [38]).

The comparison of all these data indicates that the increase in nucleic acid content and the artificial introduction of nucleic acid metabolites stimulates plant flowering, while introduction of antimetabolites, on the contrary, retards it. However, these data give little basis for the precise determination of the place and significance of substances of nucleic nature in the processes of plant flowering.

In connection with this, it seemed important to clarify two questions: 1) do these substances directly affect metabolism and morphogenesis taking place in the apical meristems, or do they have a relationship to the processes taking place in leaves; 2) are these substances capable of inducing reproductive development of short-day plants under long-day conditions when they normally remain vegetative.

METHODS AND EXPERIMENTAL CONDITIONS

For studying these problems, and in order to act directly on the metabolism of apical stem meristems, we used the method of isolated apical bud culture [39]. We have carried out five experiments in the course of 2 years from July 27, 1958, until July 15, 1960, in the laboratory of an air conditioned greenhouse of the Artificial Climate Station, Institute of Plant Physiology, USSR Academy of Sciences.

As an experimental object the short-day variety red-leafed Perilla (Perilla nankinensis) was used. The plants were cultivated in long-day or continuous light conditions and remained in a vegetative state. Then the apical buds of the main or upper side shoots were removed and grown in tissue culture conditions.

Apical buds 3 to 4 mm long were cut with the small covering leaves, thoroughly washed with soapy water and then distilled water. They were then sterilized in 0.1% mercuric chloride solution for 3 minutes and washed with 5 portions of sterile distilled water, 5 minutes in each. In sterile conditions in a special transfer room the buds were placed into test tubes (23 × 200 mm) on White's nutrient medium [40] or Gautheret's [41], which contained 2% sucrose and 0.8% agar, with addition of microelements according to Heller [42].

The test tubes with planted apical buds were placed in an air conditioned greenhouse with relative humidity of 60%, where throughout the year temperature was 22-24° in the daytime and 18-22° at night. Different light regimes were produced as follows: 1) long (16-18-hour) days were made from a natural day, to which, during autumn, winter and spring months, was added artificial light from fluorescent lamps up to 20-24 hours; 2) short (9-hour) days were made by means of lightweight cardboard chambers impenetrable to light with which test tube racks were covered for 15 hours during the day; 3) continuous darkness was obtained with these same chambers covering the tube racks throughout the experiment.

We tested mainly components of nucleic acids, both separately, and in mixtures with other components: 1) kinetin, 2) adenine, 3) adenosine, 4) guanosine, 5) cytosine, 6) mixture of nucleosides of ribonucleic acid: adenosine, guanosine, cytidine, uridine; 7) mixture of nucleosides and enzymatic hydrolyzate of casein; 8) mixture of nucleosides, casein hydrolyzate, flavinadenine-dinucleotide. Other additions were: 9) ribonuclease for lowering of nucleic metabolism rate, as well as substances which have an opposite effect of the nucleic acid derivative, insofar as their action on growth is concerned; 10) indoleacetic acid; and 11) gibberellic acid. The experiments were repeated 10 times, and each treatment consisted of 10 test tubes with apical buds.

EXPERIMENTAL RESULTS

Experiment 1. Carried out on July 27, 1958. Apical buds of the upper side branches of adult Perilla plants were put on White's nutrient medium. Experimental treatments according to the substances added to the nutrient medium: 1) kinetin (0.0005 g/liter); 2) adenine (0.001 g/liter); 3) heteroauxin (0.00005 g/liter); 4) gibberellin (0.0001 g/liter). Light conditions were: 1) short-day; 2) darkness.

Under short-day conditions the apical buds developed well, and in 5 days the cover leaves began to open and grow. Later, the stem began to grow and formation of new leaves was initiated. Formation and growth of roots took place better in the treatment with heteroauxin and weaker in case of adenine; in the case of kinetin, only large calluses were formed, and in the treatment with gibberellin neither roots nor calluses were formed.

In the darkness the buds grew and gave roots in all those cases except gibberellin; however, plants were etiolated and weak.

Differentiation of flower buds in the plants of short-day, as well as darkness, began in the treatments with adenine and kinetin: in conditions of short-day it took place on September 8, in case of adenine; on September 30, in case of kinetin; and October 5, in the cases of indoleacetic acid and gibberellin. Flower buds opened in all the treatments, but the greatest flowering was observed in the treatment with indoleacetic acid.

Conclusion of the first experiment: In conditions of short-day, favorable for Perilla flowering, as well as in the darkness, kinetin and adenine speeded up differentiation of flower buds and flowering of isolated apical buds of red-leafed Perilla.

Experiment 2. Initiated September 15, 1958. Apical buds of the upper lateral branches of the adult Perilla

plants were taken. White's nutrient medium was used. Experimental treatments included substances which were added to the medium: 1) adenine (0.001 g/liter); 2) indoleacetic acid (0.00005 g/liter); 3) gibberellin (0.0001 g/liter). Light conditions were: 1) long-day; 2) darkness.

The growth of isolated buds both in the long day and the darkness was weak; in the treatment with adenine the growth was better than in other treatments. In conditions of the long day the buds rapidly dried out and died. In darkness, differentiation of flower buds was observed October 31 only in the treatment with adenine. Also, the buds of all treatments left in the darkness died rapidly.

The conclusion of the second experiment: In darkness, adenine accelerated differentiation of flower buds in isolated buds of ref-leafed Perilla.

Experiment 3. Initiated March 14, 1959. Apical buds of main stems of young Perilla plants were placed on White's nutrient medium. Additions to the medium were: 1) control, White's medium; 2) kinetin (0.001 g/liter); 3) adenine (0.001 g/liter); 4) indoleacetic acid (0.00005 g/liter); 5) gibberellin (0.0005 g/liter). Light conditions were: 1) short-day; 2) long-day; 3) darkness.

After placing isolated buds on official nutrient medium, leaf growth began on the second or third day, and during the following 7 to 10 days growth of the shoot was observed. In that part of the plant which was submerged in the medium, a process of callus formation began. Root formation began on the 15th to 20th day after bud cultivation. At this time differences between treatments in the growth of stem, its branching, leaf coloration, size of calluses, and number of formed roots began to be clearly visible.

Plants which formed roots also formed a rapidly growing main shoot, while in the absence of roots the shoot growth was sharply retarded. The leaves which developed prior to the appearance of roots had the red coloration usual for Perilla, while the leaves of the rapidly growing shoot, which formed after root formation, were green.

In short-day conditions the growth of control plants took place sufficiently intensively; the growth was some what slower when adenine and heteroauxin were added to the medium. Introduction of gibberellin to the medium led to the retardation of callus and root formation, as a result of which the growth of the aerial part was also retarded; in the case of kinetin a considerable retardation of plant growth was observed, although callus formation was intensive. In short-day conditions in plants of all treatments – except the treatment with kinetin where growth was sharply suppressed – between the 25th and 35th day differentiation of flower buds was observed, mostly on the axillary shoots of lower leaves.

In conditions of continual darkness, control plants, as well as plants with indoleacetic acid and adenine treatments, formed a long etiolated shoot, consisting of one internode and terminating in two reduced etiolated leaves. Two lower leaves at the base of this shoot, as distinguished from the upper leaves, grew normally, but also were etiolated. Addition of kinetin and gibberellin to the medium led to the complete suppression of root formation and retardation of shoot growth connected with it. In darkness also, in all treatments except in the kinetin treatment, the differentiation of flower buds was observed in the tips of etiolated shoots 25 to 30 days after planting.

In long-day conditions, plant growth was more intensive than in short days. The most intensive growth was in control plants; somewhat weaker growth resulted from treatments with kinetin, adenine, and indoleacetic acid. In the treatment with gibberellin, as well as in the short-day and in the darkness, an intense inhibition of root formation and weak growth of aerial parts was observed (Fig. 1).

Differentiation of flower buds in long days took place, 25 to 30 days after planting, only in the treatments where kinetin and adenine were added to the medium. Formation of flower buds in these treatments, as well as in short-day conditions, was noted only in the axillary

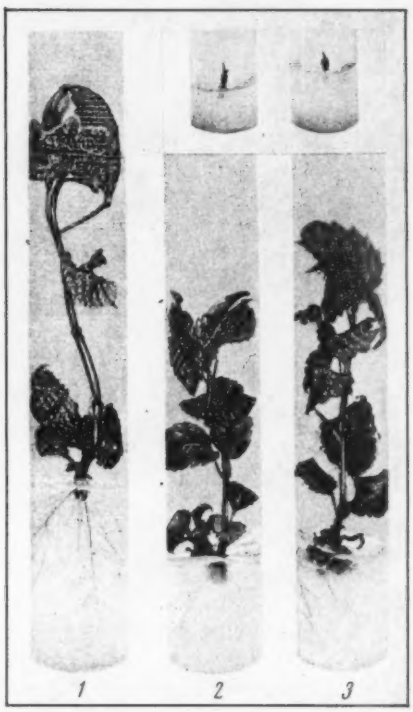

Fig. 1. Growth of red-leafed Perilla in culture in the test tubes of White's nutrient medium in long-day conditions. 1) Control; 2) adenine (0.001 g/liter); 3) kinetin (0.001 g/liter). On the upper right are apical buds of Perilla during the first day of planting (photographed June 4, 1959).

shoots of lower leaves, where with time they became clearly visible (Fig. 2).

Three months after the beginning of the short-day experiment, new flower buds appeared on the upper shoots. They grew rapidly and opened up into small flowers, while the buds on the lower shoots were retarded in their development. In plants grown in long-day conditions, no new flower buds formed at the tips of shoots, while lower flower buds stopped in their development. In both cases there was a clear effect of leaves which have been exposed to days of various lengths.

Conclusion of the third experiment: In long-day conditions when control plants were left in a vegetative state, kinetin and adenine caused differentiation of flower buds in plants of red-leafed Perilla [43]. However, differentiated flower buds did not continue development into flowers indicating an effect of leaves left in an unfavorable day length.

Experiment 4. Initiated July 27, 1959. The apical buds of two upper lateral shoots in adult Perilla plants were put on Gautheret's nutrient medium. Additions to the medium were: 1) control; Gautheret's medium, 2) kinetin (0.0005 g/liter); 3) adenine (0.001 g/liter); 4) adenosine (0.001 g/liter); 5) guanosine (0.001 g/liter); 6) cytosine (0.001 g/liter). Light treatments were: 1) short day; 2) long-day – natural day from July 27 to September 7, which grew shorter from a 16-hour day to a $13\frac{1}{2}$-hour day; then, to the end of the experiment, continuous light, made up of natural day and supplementary light of fluorescent lamp; 3) darkness.

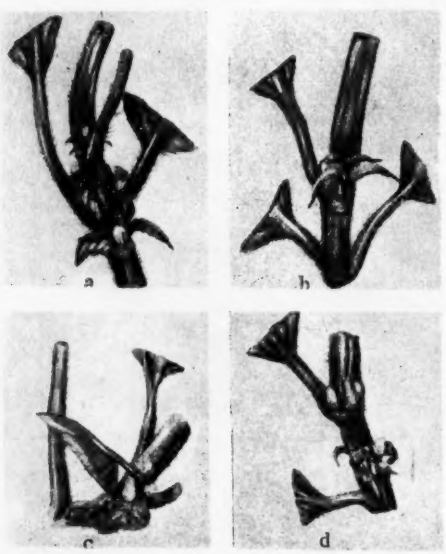

Fig. 2. Lower parts of Perilla plants grown in test tubes on White's nutrient medium. The upper row – control plants: on the left – in short-day conditions (flower buds in the axils of lower leaves); on the right – in long-day conditions (small leaves of growth shoots can be seen; no flower buds are present). Lower row – experimental plants: on the left – in long-day conditions, adenine added; on the right – the same with kinetin added (in both cases flower buds can be seen in the axils of lower leaves). Magnification 10×; drawing and photograph April 4, 1959.

In short-day conditions, substances which were added to the medium induced a certain retardation of growth, particularly significant in the case of kinetin. A month after the beginning of the experiment, by September 1, the plants of all treatments formed flower buds, but in the case of adenosine and guanosine the budding took place earlier, while in the case of cytosine it was somewhat retarded. The flowering began September 14 in the treatments with adenosine and guanosine, while the plants of other treatments still had only flower buds and began to flower later.

In long-day conditions, where the plants were also receiving an induction by a short day, the differentiation began in the treatments with kinetin, adenosine and guanosine on September 1, while the control treatments and those treated with cytosine began flowering on September 14. There was no flowering in the control treatment; in all other treatments the flowering began on October 1, and in the treatment with kinetin seed ripening was observed. With shortening natural days from July 27 to September 7 plants of all treatments reached the stage of differentiation of flower buds and that of mature flower bud formation, but in the experimental treatment the reproductive development took place at the more rapid rate. After the plants had been transferred to the continuous illumination, all shoots which had been formed in the experimental plants began to flower, and in the leaf axils flower clusters arose, while in control plants there was intensification of vegetative growth without flowering.

The derivatives of nucleic acid metabolism and kinetin, while affecting the acceleration of the reproductive development of plants, induced growth retardation, and in conditions of long day this retardation was significantly less than in conditions of the short day.

In the darkness, the plants of all treatments as usual were etiolated and formed flower buds.

Conclusion of the fourth experiment: In conditions of natural autumn day, which gradually gets shorter, followed by continuous illumination, kinetin, adenosine, guanosin, and cytosine have caused acceleration of flower bud differentiation, mature flower bud formation, and flowering, while control plants have passed the first two phases of reproductive development more slowly, and did not begin to flower.

Experiment 5. Initiated February 1, 1960. Apical buds of comparatively young plants of Perilla, planted in November, were put onto Gautheret's medium. Additions to the medium were: 1) control, Gautheret's medium; 2) kinetin (0.001 g/liter); 3) adenosine (0.002

Effect of Nucleic Acid Derivatives on Growth and Reproductive Development of Isolated Apical Buds of Red-Leafed Perilla

No.	Experimental treatment	Short day							Long day						
		differentiation of flower buds	flower bud formation	flowering	at end of experiment				differentiation of flower buds	flower bud formation	flowering	at end of experiment			
					number of plants			height, mm				number of plants			height, mm
					surviving	with flower buds	flowering					surviving	with flower buds	flowering	
1	Control, Gautheret's medium	Apr. 1	Apr. 4	Apr. 16	7	—	7	20	—	—	—	6	—	—	90
2	Kinetin	Apr. 1	Apr. 4	—	2	2	—	7	Apr. 1	Apr. 16	Apr. 13	6	5	1	70
3	Adenosine	Apr. 1	Apr. 4	Apr. 4	5	1	4	10	Apr. 19	Apr. 23	May 3	4	2	2	90
4	Guanosine	Apr. 1	Apr. 16	Apr. 3	2	—	2	20	Apr. 16	—	—	3	3	—	90
5	Cytosine	Apr. 1	Apr. 11	Apr. 16	2	—	2	20	Apr. 11	Apr. 28	—	2	2	—	80
6	Nucleoside mixture	Apr. 1	Apr. 4	Apr. 11	5	—	5	15	Apr. 1	Apr. 11	May 16	5	4	1	90
7	Nucleoside mixture and casein hydrolyzate	Apr. 1	Apr. 4	Apr. 7	6	—	6	10	Apr. 1	Apr. 16	May 3	6	1	5	90
8	Nucleoside mixture, casein hydrolyzate, and flavin-adenine-dinucleotide	Apr. 1	Apr. 4	Apr. 11	4	1	3	40	Apr. 1	Apr. 16	—	5	2	—	130
9	Ribonuclease	Mar. 23	Apr. 4	Apr. 4	5	—	5	20	Apr. 1	—	—	2	2	—	110

g/liter); 4) guanosine(0.002 g/liter); 5) cytosine (0.002 g/liter); 6) mixture of ribonucleic acid nucleosides (RAN): adenosine (0.0005 g/liter), guanosin (0.0005 g/liter), cytidine (0.0005 g/liter), uridine (0.0005 g/liter); 7) mixture of nucleosides RAN and casein hydrolyzate (0.4 g/liter); 8) mixture of nucleosides RAN, casein hydrolyzate, and flavin-adenine-dinucleotide (0.002 g/liter); and 9) ribonuclease (0.01 g/liter). Light conditions were: 1) short day; 2) long day.

Growth of apical buds and Perilla plants took place in conditions of both short and long day at a sufficient rate, although with great differences in various experimental treatments. In the table, the data are given on the reproductive development and growth of plants according to the light conditions and effect of the tested substances.

In short-day conditions, there was considerably greater growth of vegetative organs in experimental plants which had been given a mixture of nucleosides, casein hydrolyzate, and flavin-adenine-dinucleotide (treatment 8) than in the control plants. On the other hand, plant growth was retarded in the treatments with adenosine (treatment 3) and the nucleoside mixture with casein hydrolyzate (treatment 7); particularly sharp growth retardation was shown with kinetin (treatment 2).

Differentiation of flower buds in most treatments, control treatments among them, began on April 1; in the treatment with ribonuclease it began earlier – March 24. Flower bud formation in all treatments began April 4, except for treatments with guanosine and cytosine, where it was somewhat retarded. Simultaneously on

Fig. 3

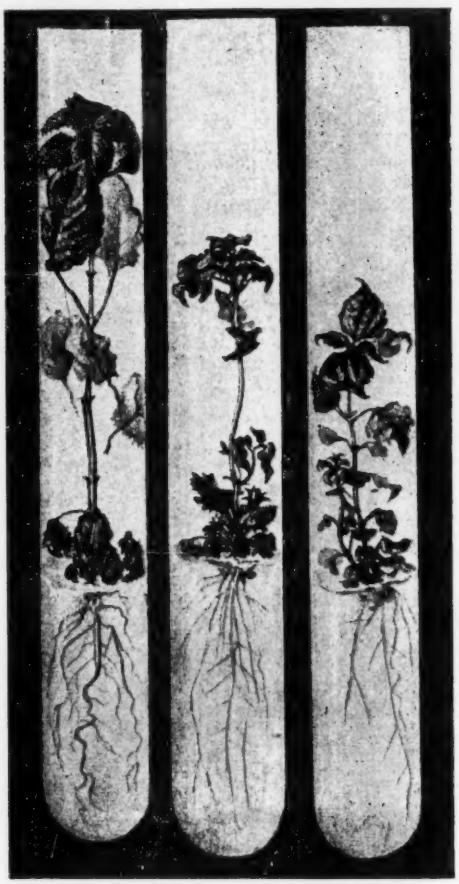

Fig. 4

Fig. 3. Effect of day length on growth and development on plants of red-leafed Perilla, grown from isolated apical buds in test tubes on Gautheret's nutrient medium. Left: long day, the plant is in vegetative state; right; short day, new plant flowers and forms fruit (drawing and photograph June 15, 1960).

Fig. 4. Effect of nucleic acid derivatives on growth and development of Perilla plants in test tubes on Gautheret's medium in long-day conditions. On the left: control, the plant is in vegetative state; in the middle: adenosine (0.002 g/liter); on the right: kinetin (0.001 g/liter); in both cases the plants flower (drawing and photograph June 15, 1960).

April 4, the plant flowering began in the treatment with adenosine, ribonuclease, and somewhat later (even though earlier than in control plants) in the treatment with nucleoside mixture (treatments 6, 7, 8). The plants in the treatment with cytosine flowered simultaneously with controls, in the treatment with guanosine considerably later, and in the treatment with kinetin did not flower at all, due to the general depression of growth processes. In the first week of May all flowering samples had formed seeds.

In long-day conditions, growth of plants in all treatments in general was considerably more rapid than in conditions of short day. The greatest growth again was shown by the plants with the treatment that included the mixture of nucleoside, casein hydrolyzate, and

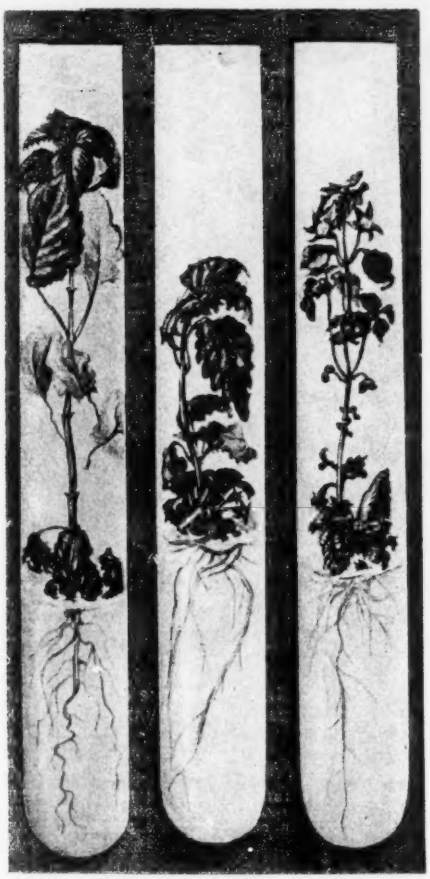

Fig. 5. Effect of nucleic acid derivatives on the growth and development of Perilla plants, in test tubes on Gautheret's medium in long-day conditions. On the left: control, plant is in vegetative state; in the middle: mixture of ribonucleic acid nucleosides (0.002 g/liter); on the right: the same mixture and casein hydrolyzate (0.4 g/liter); in both cases plants flower (drawing and photograph June 15, 1960).

flavin-adenine-dinucleotide (treatment 8), as well as treatments with ribonuclease (treatment 9). The greatest growth retardation was shown by kinetin, and somewhat lesser retardation by cytosine. In long-day conditions, under influence of products of nucleic acid metabolism, the changes in form and coloration of leaves arose: deformation and thickening of the leaf blade took place, the leaf edges grew together to form bell-like structures (phyllode), and intense anthocyanin coloration appeared.

In long-day conditions, control plants remained to the end of the experiment in the reproductive growth phase and did not pass into the differentiation of flower buds (Fig. 3).

Plants of all other treatments at different times initiated flower buds, but mature flower buds were absent in treatments with guanosine and ribonuclease, while flowering took place only in the treatments with kinetin, adenosine, nucleoside mixture and the same mixture supplemented by casein hydrolyzate (treatments 2,3,6,7). The plants treated with adenosine began to flower very rapidly and the plants treated with kinetin somewhat more slowly (Fig. 4).

The flowering of plants was particularly stimulated by the mixture of ribonucleic acid nucleosides, and even more when the same mixture was supplemented by casein hydrolyzate (Fig. 5). Addition of flavin-adenine-dinucleotide to this mixture caused intensification of vegetative growth and retardation of development for already-initiated flower buds.

The effect of ribonuclease on the development of plants turns out to be closely related to the length of day – in short-day conditions ribonuclease considerably stimulated flower bud formation and flowering of plants, while in long-day conditions ribonucelase induced differentiation of flower buds, but then inhibited their development into the mature buds and flowers (Fig. 6).

Conclusion of the fifth experiment: In long-day conditions where control plants remained vegetative, the products of nucleic acid metabolism – adenosine, guanosine, and cytosine – mixture of nucleosides both taken separately as well as with casein hydrolyzate, as well as kinetin in a different degree, induced the reproductive development of red-leaved Perilla plants. In the same conditions, ribonuclease at first induced initiation of flower buds, later retarded their development while stimulating the process of vegetative growth.

CONCLUSION

The data given in the present work indicate that the culture of isolated apical buds may be successfully used for clarification of the physiological nature of processes inducing change of stem buds from vegetative growth to reproductive growth. The advantage of this method over the usual vegetative methods is the possibility of continued action on the growing apical points by any organic compounds introduced into the substrate in sterile conditions, which prevents their breakdown by microorganisms and makes possible the activity of this

substance directly through the cut surface of the bud, leaving out root and stem. In the development of plants arising from apical buds in test tube culture, two periods are observed. In the first period the plant forms only several leaves, and since it does not yet have a root or rapidly growing stem, it is altogether acted upon more directly by the substances of the nutrient medium. During the second period the plant forms the root, rapidly growing shoot, and numerous leaves, and therefore the effect of the substances from the nutrient medium becomes less pronounced, while the effect of leaf assimilants becomes predominant.

Turning directly to the questions introduced at the beginning of this study, we may consider that the first question may be given a reasonable solution. The data in our work indicate that substances related to nucleic acids introduced into the substrate act directly on metabolism taking place in the stem bud meristem, since their effect is shown even in those cases where no root is formed, and the growth of the shoot and newly formed leaves takes place very slowly. However, these data do not resolve the question as to what relationship exists between processes taking place in leaves and changes in the growth points induced by these nucleic acid derivatives introduced into the nutrient medium.

Insofar as the second question is concerned, it is resolved more completely. The derivatives of nucleic acid metabolism, and in particular the substances of purine and pyrimidine type, not only stimulate the flowering process in a typical short-day variety – red-leafed Perilla – in short-day and in continuous darkness, but also induce it in long-day conditions, when control plants remain in a

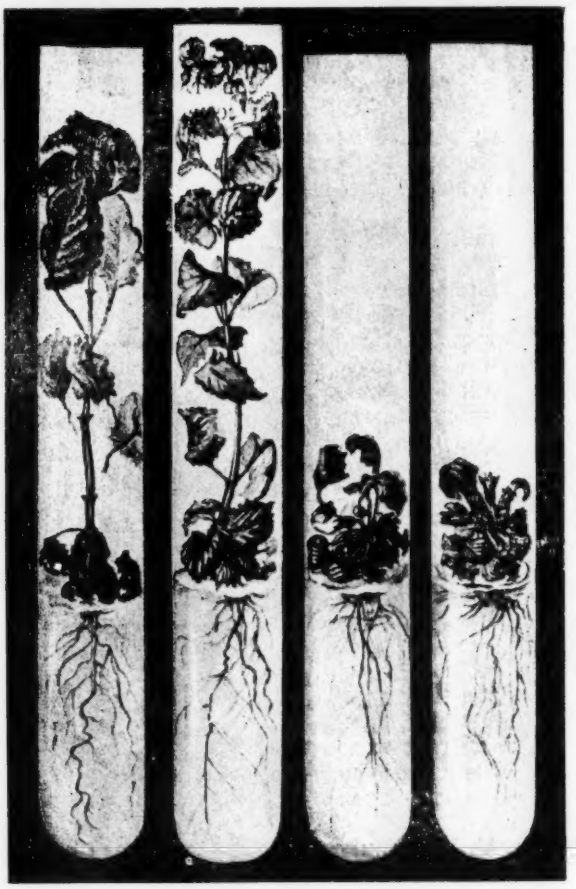

Fig. 6. Effect of the enzyme ribonuclease on growth and development of Perilla plants in test tubes, on Gautheret's medium, in long and short days. From left to right: control on long days (plant is in vegetative state); ribonuclease (0.01 g /liter) on long days (plant is in vegetative state, with flower buds in the axils of lower leaves); control on short day; ribonuclease (0.01 g/liter) on short day. In both cases plants flower and bear fruit (drawn and photographed June 15, 1960).

vegetative state. Here the most effective of various substances are kinetin and adenosine, but the nucleosides of ribonucleic acid are even more effective, especially when supplemented by casein hydrolyzate. At the same time these substances and their mixtures induce retardation of vegetative growth, i.e., they act opposite to indoleacetic acid and gibberellin, which usually intensify vegetative growth but are not able to induce reproductive development in a short-day variety.

Synergism in the action of the nucleoside mixture indicates that none of the substances studied have a predominant significance, since all of them affect the same process. This is also indicated by the observation that the enzyme ribonuclease, which breaks down ribonucleic acid when included in the medium, weakens the reproductive development and increases growth, and flavin-adenine-dinucleotide acts in the same direction.

At the present time it is difficult to give a completely definite answer with respect to the nature of those changes in metabolism which are induced by introducing into plants the derivatives of nucleic acid metabolism, particularly purine and pyrimidine. However, there is a basis for supposing that this intensifies synthesis of nucleic acids and proteins. In a number of investigations it has been shown that kinetin considerably increases the synthesis of deoxyribonucleic acid and proteins in plant tissues [44-47]. In our experiments, when kinetin and adenine were added to the nutrient medium the content of proteins was increased and content of soluble sugars was decreased in the tissues of isolated apical stems.

The significance of nucleic acids and their derivatives in the activity of plants, in their growth and development, every year becomes more clear and more widely known. Many years ago Sabinin [48] for the first time proposed in a large scheme their great significance in growth processes, and recently many investigators are beginning to assign them a primary significance in the process of plant flowering (Lona [49], Bonner [50], Lang [51], Nakajama [52]).

The experimental data and theoretical considerations given in this paper seem to us to give a new basis to the consideration of the significant role of substances related to nucleic acids in the flowering of short-day plants.

SUMMARY

1. The derivatives of nucleic acid metabolism, particularly substances of the purine and pyrimidine type, stimulate the flowering process of isolated shoot apices and plants of red-leafed Perilla developing from them, both in short-day conditions as well as in darkness. Furthermore, they induce flowering in long-day conditions, both in the presence as well as in the complete absence of photoperiodic induction, while control plants remain vegetative.

2. No single substance of a nucleic acid nature has a predominant specific action. The most effective of the substances affecting flowering are kinetin and adenosine; still more definite is the action of the mixture of nucleosides of ribonucleic acid, particularly in those cases when this mixture is supplemented by casein hydrolyzate. Addition of ribonuclease to the medium leads to retardation of the reproductive development and the increase of growth. The same action is shown by flavin-adenine-dinucleotide.

3. Derivatives of nucleic acid metabolism—purines, pyrimidines, nucleosides—when introduced into the nutrient medium first and foremost affect the metabolism in the meristems of stem buds. It is most probable that in the plant tissues the content of nucleic acid and proteins is increased, contributing to the change of short-day plants from vegetative to reproductive development.

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ON THE VITALITY OF SECTIONS OF LEMON LEAVES

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In this communication we should like to call attention to an interesting property of lemon leaves accidentally detected by us. As previously stated [1], we were determining the photosynthetic capacity of mature lemon leaves which had been frozen for various lengths of time. For this purpose discs of 10.2 cm^2 were cut from thawed leaves and their photosynthetic intensity measured in a Warburg apparatus. Subsequently 3 of these discs were placed in Petri dishes, the bottom and top of which were covered by moist filter paper. The dishes were left in a laboratory under diffuse light. The same was done with discs from control plants which were not frozen.

We noticed that the cut-outs from control leaves, as well as the ones not damaged by freezing, had a perfectly normal appearance one to two weeks and even 4 months after they were placed in Petri dishes. In the same study [1] results are given of photosynthesis intensity determinations, showing that it remained at approximately the same level for the indicated period. We have since become interested in similar experiments with other plants (for instance, cabbage, cucumbers), cut-outs from whose leaves began to lose the green coloration in a few days and subsequently decayed.

We decided to leave the cut-outs of lemon leaves in Petri dishes for a longer period of time. The only care taken was that the paper in the Petri dishes should not become dry, and periodically we moistened the paper with tap water without adding any other substances.

The first batch of 12 cut-outs was placed in Petri dishes in October, 1953. They remained without any change for a year and then an additional new batch of 30 cut-outs was placed in 10 dishes. The majority of the cut-outs of both batches remained in the dishes until the fall of 1959, i.e., for 5-6 years; a portion of the cut-outs decayed during that time. Only during the winter of 1959-60 did the decay of most cut-outs begin; this was caused by insufficient care and not because of aging, since cut-outs from 1953 as well as 1954 were preserved until the winter of 1959-60. Probably with attentive care these cut-outs could have lasted a few more years.

Variations by Years of Photosynthesis Intensity of Cut-outs from Lemon Leaves (mg CO_2/hr/dm^2)

Experiment number	Year of determination			
	1953	1954	1956	1957
1	4.8	8.3	7.5	—
2	10.6	11.2	6.2	4.3
3	—	4.9	4.7	3.9
4	—	6.1	6.9	5.5

As a control of the state of the cut-outs, the photosynthesis intensity of several was measured once a year in a Warburg apparatus, i.e., under identical illumination and temperature conditions. Results of these determinations are shown in the table. Data of 4 experiments are given; in each experiment there were 3 cut-outs (from one leaf); the figures in the table are the means of photosynthesis determinations for the 3 cut-outs.

It can be seen from the data in the table that over a 3-to-4-year period the photosynthetic capacity of the cut-outs varied little, which indicates their normal physiological state. Unfortunately, no determination of photosynthesis of these cut-outs was conducted during the last 2 years. It is interesting that in the first year of the experiment the photosynthetic intensity of the cut-outs did not decrease, and increased in some cases, despite the absence of outflow of assimilants. The cut-outs at the time preserved their normal green appearance. It is known from the literature, however, that when outflow of assimilants is discontinued from lemon leaves on the tree (on ringing branches) a decrease in green coloration of leaves becomes evident even in a few weeks [2, 3].

The preservation of a normal green coloration of the cut-outs and normal photosynthesis in our experiment is probably explained by the fact that the cut-outs were not subjected to action of strong light; therefore, the assimilants in them could not have been accumulated to an excess, even in the absence of outflow, and consequently the cut-outs did not decrease their photosynthesis intensity and did not stimulate chlorophyll destruction. We could not study the carbohydrate content in the cut-outs and the effect of illumination of

varied intensity on the state of the cut-outs. It is possible that, if kept brightly illuminated, they would have soon lost their green coloration and died. Observations by Nekrasova [3] confirm that chlorophyll destruction in leaves of ringed branches proceeds considerably faster in bright light (in summer environments compared to winter ones). On preserving the cut-outs in even a weaker light or in darkness, their destruction would probably have also occurred much sooner, due to exhaustion and expenditure of organic substances in the respiration process.

Our observations permit some conclusions of a general character. It is known that lemon leaves on trees usually live for 2 years. However, our experiments show that detached lemon leaves (cut-outs from them) can live 6 years, and probably longer. This becomes more interesting because it was repeatedly noted in the literature that leaves detached from plants usually turn yellow faster than those left on the plant [4]. Probably the rapid aging of lemon leaves on the tree is related either to the effect on them of other organs or portions of the plant, or to the effect of unfavorable external environments to which leaves on the tree are subjected (for instance, bright illumination, air aridity). Moreover, our experiments show that a prolonged life of leaf tissues is possible without external intake of any substances (mineral or organic). Apparently, cells of adult lemon leaves possess a sufficient store of mineral substances necessary for life, while the necessary organic substances are created by them in the process of photosynthesis.

The question arises then: what accounts for such a prolonged survival of cut-outs from lemon leaves as contrasted to cut-outs from leaves of other plants (for instance, of cabbage, cucumber) which decay even after one week? Possibly this is related to the presence of essential oils in lemon leaves which can exert a phytocidal effect on different microorganisms. But, evidently, this alone is not the case. As we have noted earlier, the chlorophyll is rapidly destroyed in cut-outs from leaves of other plants, and they turn yellow, while in cut-outs from lemon leaves, even if there is destruction of chlorophyll on prolonged preservation, it proceeds very slowly. Consequently, the chlorophyll-protein complex in lemon leaves is endowed with great resistance in environments of weak illumination and freedom from effects through other organs. It would be interesting to ascertain whether this feature is also characteristic of other perpetually green plants.

It seems to us that the conclusions arrived at here and the questions raised touch upon important aspects of plant physiology, and therefore a further examination and study are necessary. Moreover, cut-outs from lemon leaves and, possibly, other plants capable of preserving their normal state for a prolonged period of time, may become convenient objects for studying various problems of metabolism in leaves, especially in cases when slowly moving processes are studied which require a course of prolonged experimentation. Cut-outs from leaves of other plants (for instance, turnip, cabbage, beans, squash) have already been utilized for a study of their ability to synthesize folic and ascorbic acids [5, 6], but the experiments lasted only 2-3 days.

Cut-outs from lemon leaves and some other plants can probably be used to study synthetic paths of various compounds in plants by placing these cut-outs in solutions of different substances. They can be used for the study of effects of different substances on processes of respiration and photosynthesis; for tissue resistance to various unfavorable factors (for instance, to frost).

In short, we think that such cut-outs from leaves can become convenient objects for solving a number of physiological problems of organs and tissues which have completed their growth. The convenience of this object, apart from its protracted life period, also consists of the fact that such cut-outs do not require complex nutrient media or sterile conditions to support life as is the case in artificial cultures of other plant organs and tissues.

SUMMARY

Sections of lemon leaves kept in a moist chamber under weak illumination retained their vitality during a period of six years. Possible explanations of this phenomenon are considered. It is suggested that this peculiarity of lemon leaves may be used in various physiological experiments.

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BRIEF COMMUNICATIONS

CARBOHYDRATE METABOLISM IN WILD CHINESE APPLE TREES DURING THE PERIOD AFTER PLANTING AND ITS IMPORTANCE FOR THEIR SUBSEQUENT GROWTH

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Carbohydrates, together with amino acids, nucleic acids and proteins, are the main materials required by the plant for the renewal of its cells, tissues and organs and for regenerative processes as a whole [1-5, etc.].

The present communication deals with a research project involving the study of certain peculiarities in the carbohydrate metabolism of wild Chinese apple trees during the period immediately after planting. These peculiarities of metabolism have a direct influence on the regenerative processes following planting and determine the rate at which the latter occur. Consequently they have a marked effect on the capacity of the tree to acclimatize itself.

The research was carried out during 1956-1957 at the "15 let Oktyabrya" State Fruit Farm in the Saratov Region. Before planting, the experimental field was treated with manure at the rate of 30 tons per hectare and then cultivated to a depth of 40-50 cm. Autumn planting of the wild stock took place in the middle of October, spring planting at the end of April. The aerial portion of the stock was shortened to half its former length after the plants had been transferred to their new quarters. Routine care of the wild stocks included weeding, hoeing and twice watering the field at a rate of 300-400m^3/hectare. Tests were performed 10-20 days after planting, when the carbohydrate was estimated by the Haagedorn-Jennsen method. Phenological observations and growth measurements were made at regular intervals. Root growth was determined by the Kazakevich-Hiver trenching method.

Consumption of carbohydrate by the plant is a somewhat complex process and it was not possible to relate this process with the formation of new organs in all cases (c. f. figure). Nevertheless, the carbohydrate consumption of many newly-formed organs was found to be very high indeed and certain parts of the plant consumed two-thirds of their available carbohydrate reserve in this manner. Thus, the carbohydrate content of the median portion of the shoot dropped from 14.5% to 6.1% in a short period of time (between May 22 and June 9). This diminution in carbohydrate was mainly due to starch being used in the metabolism of the organs concerned.

In the lower portion of the shoot the minimum carbohydrate content was recorded at the time of the mass appearance of the leaflets (May 22). It would appear that the formation of the young leaves is accompanied by an intensive hydrolysis of starch and active translocation of the resultant sugars to the site of leaf formation. This hypothesis is confirmed by the quantitative relationship between sugars and starch in the median and lower parts of the shoot at the first time of sampling; of particular significance is the fact that, whereas the monosaccharide and disaccharide contents of these parts of the shoot are approximately equal, there is a considerable difference in the starch content (7.72% as compared with 2.86%).

The carbohydrates of the root are also consumed in the regenerative processes. This is manifested by the diminution in amounts of starch and disaccharide sugars. In particular, the disaccharide content of the root fell from 3.99% to 0.80% between May 22 and June 9.

The quantity of soluble carbohydrate (monosaccharides and disaccharides) in both stems and roots undergoes a marked fluctuation during the course of the vegetative period, as a result of these substances being implicated in a wide variety of biochemical transformations. However, attention should be paid to the high disaccharide content of the shoot (experiment of 1956, June 1 determination) at the beginning of linear growth renewal of the stem. An increase in the content of disaccharides occurs in the period of maximum growth of stems and roots (experiment of 1956, July 9 determination).

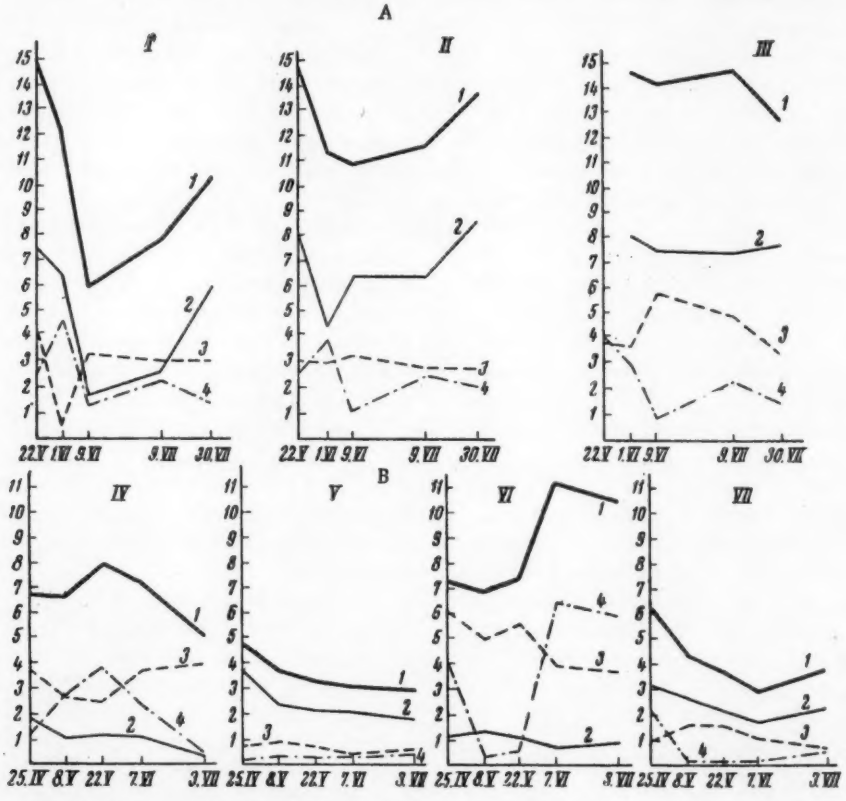
During that particular period after planting when the diminution in carbohydrate content of plants occurs,

The Bursting of Buds in Wild Apple Tree Stocks Transplanted in the Autumn and their Carbohydrate Content (expressed as % of absolute dry wt.). Data calculated from sampling on April 24, 1957

Mono-saccha-rides	Di-saccha-rides	Starch	Total carbo-hydrates	Comments
1.78	2.84	0.89	5.51	wild stock not transplanted, buds dormant
2.89	0.26	0.74	3.89	stock transplanted in autumn, buds dormant
2.09	8.54	1.81	12.37	stock transplanted in autumn, buds bursting into growth

the regenerative processes form new leaves, new shoots and new roots. It is, therefore, of considerable interest to determine the precise destination of the carbohydrate which is lost from the reserves. The figures relating to carbohydrate levels in the median portion of the shoot indicate that the mass breakdown of starch and disaccharides coincides with the commencement of linear growth in the young branches. Consequently, it would appear that the bulk of the carbohydrate reserve goes into the formation of young branches and offshoots.

It is obvious that the regenerative processes use a large amount of carbohydrate. The maximum diminution in levels of both starch and sugars takes place at the time when young shoots are forming on the median portion of the apple tree stocks. This implies a definite localization of carbohydrate consumption within the



Carbohydrate content (as % of the absolute dry weight) in wild apple trees during the period after planting. A) Expt. 1956 (April 28 planting); I) Median portion of shoot; II) lower portion of shoot; III) main root; B) Expt. 1957 (April 22 planting); IV) cortex of median part of shoot; V) woody part of median portion of shoot; VI) cortex of main root; VII) woody part of main root. 1) Total carbohydrate; 2) starch; 3) monosaccharide; 4) disaccharide.

plant, corresponding to the site of formation of the new shoots.

The diminution in carbohydrates among newly-planted wild apple trees continues until the first half of July. After that, carbohydrate metabolism changes from being mainly degradative to being partially synthetic, with a consequent accumulation of starch. Thus, from the time of autumn-lifting until July, the wild stocks have been steadily using up their carbohydrate reserves and only after a very long interval of time (eight-ten months) does a reversal of this process take place and do the stocks begin to attain a positive carbohydrate balance.

The period of soluble carbohydrate accumulation in the buds (see table) is an important phase in the sequence of changes which follow transplanting.

It is obvious from the table that the opening buds contain several times the amount of sugar present in the same buds when dormant. Disaccharide sugars constitute the largest fraction of the soluble carbohydrate present in wakening buds.

It is interesting to note that the buds on non-transplanted stocks contain a greater percentage of carbohydrate than those of stocks which were transplanted in the autumn and in which the buds are still dormant. During the winter a considerable amount of water is lost from stocks of the autumn planting and this, no doubt, hinders the translocation of carbohydrate to the buds. The actual figures for the water content of the buds on April 24, 1957, are 64.5% in the case of bursting buds and 43.5% in the case of buds which had not commenced to open. In extreme cases this loss of water from the stocks reaches a point where the plants perish.

The presence of a slight amount of growth in the shoot or root of wild stocks (plants with single newly-formed rootlets or isolated bursting buds) causes their sugar content to be higher than that of stocks in which growth is entirely absent. It would appear, therefore, that for the commencement of growth it is necessary that the total carbohydrate content of stem and root should be of a definite value and that the relative amounts of the various forms of carbohydrate should attain their appropriate values.

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EXCHANGE OF SUBSTANCES BETWEEN NEIGHBORING HIGHER PLANTS AND SOIL ALGAE

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The exchange of substances between neighboring higher plants, between higher plants and soil algae and other microorganisms is based on the presence in plants of root secretions [1-11]; they compose 10% of the dry weight of the growing plant [3].

The study of the use of root secretions by higher plants through the medium of microbes has been neglected. We have studied the interaction between plants with a common habitat, as well as the interaction between plants and soil algae.

The plant received radioactive phosphorus (P^{32}) or radioactive sulfur (S^{35}) through the leaves in all experiments. A thin layer of absorbent cotton was placed on the leaf; it was then moistened with a radioactive solution from a pipette several times during the day. Foliar feeding continued from 1-10 days. The following plants were used in the experiments: corn, millet, rice and peas.

Experiment 1. Water culture. Shoots of corn in the three or four leaf stage were used in the experiments. Four plants were placed in a vessel containing Hellriegel's nutrient solution. Two of them received leaf feeding with a solution of radioactive methionine used to wet the leaves. For the first eight days the activity of this solution was 6 microcuries/ml, then 40 microcuries for 5 days. Two plants were treated with water. Analysis of the amount of radioactive sulfur in the leaves and roots both of the fed and of the neighboring (unfed) plants was carried out 19 days from the beginning of the foliar feeding. The experiment was repeated 5 times. Representative samples were taken for analysis. Results of the experiment are presented in Table 1.

From Table 1 it is seen that the radiosulfur is found in the shoots of corn not receiving S^{35} but growing in the same vessel with plants being fed through the leaves with radioactive isotope.

Experiment 2. Soil culture. Corn was cultivated in crystallizing dishes with soil and sand (upper layer) from the three- or four-leaf stage. The number of cultivated plants in each basin, among them the plants fed with methionine, are seen from the following data:

No. of crystallizing dish	Number of plants	Fed with methionine
I	8	6
II	7	5
III	5	4

The leaves were treated with radioactive methionine for 6 days.

TABLE 1. Radioactivity of Separate Parts of Plants Receiving Foliar Feeding of S^{35}, and Neighboring Plants Not Receiving S^{35} Feeding (counts/min/100 mg wet plant tissue)

Expt. No.	Plant	Plant, receiving S^{35}				Neighboring plant, not receiving S^{35}			
		leaves	roots	anthers	seeds	leaves	roots	anthers	seeds
1	Corn	1787	500	—	—	18	6	—	—
2	Corn*	5860	336	—	—	743	120	—	—
		11250	—	—	—	540	—	—	—
		7423	341	—	—	18	6	—	—
3	Corn	365	—	526	696	0	—	32	32
		—	—	3300	—	—	—	111	—
	Rice	15461	—	—	—	73	—	—	—
		16328	—	—	1345	51	—	—	42
	Millet	745	—	67887	—	18	—	608	—

* In the second experiment the plants were cultivated in crystallizing dishes.

TABLE 2. Radioactivity of Roots and Leaves of Experimental Plants Receiving Leaf Feeding with S^{35} and Algae, Placed in Water Culture (counts/min/ /100 mg wet tissue)

Expt. No.	Experimental plant	Leaves	Roots	Algae	In percent of the activity in the leaves
5	Corn	8446	3903	1800	21.3
6	Peas	7592	583	473	6.2

The activity of the solution was 50 microcuries/ml. Analysis of the S^{35}-fed and neighboring (unfed) plants was carried out for 7 days after feeding. A representative sample from the plants of each vessel was taken for analysis.

Experiment 3. Soil culture. Plants were grown in containers till the projection of the heads (see Table 1). There were two corn plants in Vessel No. 1 and three in Vessel No. 11; for rice, two and five, respectively; and for millet, four. One of the plants in the vessel received foliar feeding with radioactive substances: corn, $K_2HP^{32}O_4$ (activity of the solution 7.2 microcuries/ml); millet and rice, radioactive methionine (activity 27.8 microcuries/ml). The leaf feeding was carried out from the beginning of the projection of the head and lasted three to four days. To avoid contamination of the neighboring plants by radioactive substances the heads of plants receiving leaf feeding were closed off with parchment.

Analysis of the leaves, anthers, and seeds of corn were carried out at 14 days and of millet and rice at 8 days from the beginning of feeding (see Table 1). Thus, it was possible for neighboring plants while carrying on their life processes to exchange nutritive substances between themselves.

If we focus attention on the transmission of nutritive substances in a single direction from the plants receiving foliar nutrition to plants not receiving the latter (in reality the process is reversible), then in percent this is expressed in the following way: corn—leaves from 1 to 13.7%; roots from 19.4 to 49.9%; anthers from 3.3 to 6%; seeds, 4.6%. Rice—leaves from 0.3 to 0.4%; seeds, 3.1%. Millet—leaves, 2.4%; anthers, 0.9%.

In spite of the short duration of the experiment (7-14 days) it is obvious that plants with common habitat transfer significant quantities of nutritive substances from one to another. It can be assumed that during the entire growing period the plants transfer even greater amounts of nutritive substances from one to another.

Experiment 4. In glass vessels containing nutrient medium other vessels were placed which had a smaller diameter, made from foil and having very fine apertures. Two 10-day-old shoots of corn were placed in the smaller vessels. Between the walls of the inner and outer vessels the plants rice and corn were placed; this guaranteed the absence of contact between the root systems. The plants in the inner vessel received leaf feeding with S^{35}. Analysis of the plants placed between the walls of the vessels showed significant amounts of radioactive sulfur.

Consequently, migration of substances from plant to plant takes place, and, in this case, when their root systems are not in contact.

Experiment 5. The investigation was carried out in water culture on 10-day-old shoots of corn and peas. Two plants were placed in flasks. A culture of soil algae was introduced into the medium. Both plants received leaf feeding with radioactive methionine for 9 days. The activity was 40 microcuries/ml. Ten days after beginning the feeding both the plants and the algae were analyzed (Table 2).

From the data of Table 2 it is seen that algae, actively absorbing root secretions, accumulate significant amounts of radiosulfur. The activity of the nutrient medium consisted of less than 1 count/min per 100 ml; that is, practically all the root secretion was captured by the algae; they contained 6.2 and 21.3% of the amount of radioactive sulfur applied to the leaves.

Experiment 6. A washed culture of algae, taken from experiment 5 (1800 counts/min/100 ml), was carefully placed in flasks with 300 ml nutrient solution. Ten-day-old shoots of corn were then placed one in each flask. After 10 days the same young leaves of corn were analyzed. The sample was the average from three vessels. The results of the experiment showed the following: leaves had a radioactivity of 163 counts/min/100 ml dry tissue; that is, they received about 9% of the radioactive sulfur from the algae. Obviously, the soil algae, as do other soil microorganisms, return to the plants part of the root secretions they take up. This does not exclude the possibility that part of the root secretion is returned to the plants through chemical transfer without the participation of the soil microbes.

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TRANSPIRATION IN PINE TREES OF VARIOUS SPECIES SHOWING NATURAL HYBRID GRAFTING

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Cases of trees belonging to different species manifesting coalescence of growth are very rare. Only a few isolated cases of this phenomenon are given in the literature and in the majority of them the phenomenon is merely recorded and not studied.

There is a radical difference between the cases in which the growth coalescence involves trees of the same species as compared with others in which two different species are involved. The former situation is a widespread natural phenomenon of regular occurrence; the latter is uncommon, exceptional and fortuitous. This may be explained by the fact that under normal conditions plants belonging to one species find it difficult to assimilate foreign organic substances elaborated by plants of a different species. Consequently, even if such plants come into close proximity, fusion of their growth (self-grafting) does not take place. It is widely known that if two plants of different species are grafted together so that both components remain on their own roots, then coalescence of their respective tissues does not take place. Coalescence only occurs if one of the components of such a union, gradually and over a long interval of time (sometimes only after several months) becomes separated from its own root system. Coalescence of this kind is of an almost obligatory nature, for if it does not occur, then the rootless component inevitably perishes.

Such a case of compulsive fusion of tissues may occur in nature. There are, for example, several descriptions in the literature of the coalescence of growth between the common pine (Pinus sylvestris L.) and the Siberian cedar (Pinus sibirica Mayr.) [1-4]. F. A. Solov'ev, after carrying out detailed examinations of such cases, makes the following comment, "A cedar seed, shed from its parent tree, must have been hidden by some squirrel or chipmunk in a crack or damaged part of the pine tree. The seed must then have germinated in this position and its tissues have become fused with the living tissues of the pine, such that ultimately the cedar branch derived its nourishment from the pine stock" [4]. It is obvious that if the growing cedar seedling had not achieved a coalescence of its tissues with the pine, it would have perished. F. A. Solov'ev mentions that the cedar branch of the compound tree has short needles, smaller pines, etc. The cedar seeds from cones borne on the cedar-pine "hybrid" have the taste of pine seeds. F. A. Solov'ev considers that all the reported cases are examples of naturally occurring vegetative hybrids (chimaeras).

Several other similar cases of coalescence between different species have already been described in the literature and will not be dealt with here [5-7].

The Kamyshinskii Forest Development Center seemed a particularly likely place at which this phenomenon of interspecific coalescence might occur. This presumption was made on the basis of the presence of mixed plantations of introduced trees of various species belonging to a single genus and also because at this center the plantations are particularly dense.

We discovered twelve cases of coalescence between the common pine and the Crimean pine, six cases of coalescence between the common pine and Bank's pine (Pinus banksiana) and single cases of coalescence between the common pine and the resin pine, Tatarsk maple and field maple, holly maple and Lobel's maple, holly maple and silver maple and between feathery elm and English elm. All these examples were more or less similar to those described in the author's previous articles [8-10]. In this present communication, it is our intention to explain the results of our investigations into the daily rhythm of transpiration in interspecific "coalescence hybrids" of various pine trees (Crimean/common pine and Bank's/common pine coalescence hybrids). The investigations were carried out using rapid weighing techniques, with five repeats and three minute exposures. The water loss was determined by means of Tiren's formula and expressed as volume lost per unit area of leaf surface. (A description of the method used is given in a previous paper by the author [10]).

Common pine/Crimean pine "coalescence hybrids" were discovered by the author in a mixed plantation of 45 years' growth. The plantation consisted of about 70% common pine and 30% Crimean pine trees. Density: 0.7-0.8.

Characteristics of Pine "Coalescence Hybrids" and Normal Trees (controls)

Components	Height, m	Diameter, cm		Projection of crown		Height of crown	Previous year's growth in height, cm
		at base	at 1.3 m height	N–S	E–W		
Common and Crimean pine							
Common pine:							
coalescence hybrid	11.5	34	27	5	6	4.0	12
control	10.6	30	23	4	4	4.0	6
Crimean pine:							
coalescence hybrid	10.4	32	25	4	5	5.0	10
control	7.9	27	19	4	4	2.5	6
Common and Bank's pine							
Common pine:							
No. 1– coalescence hybrid	9.7	24	19	3.0	3.7	3.0	6
No. 2– coalescence hybrid	9.9	31	25	3.5	4.0	2.5	6
Control	5.8	32	27	5.0	5.0	2.0	1
Bank's pine:							
coalescence hybrid	7.5	32	28	5.0	7.0	1.8	6
control	3.3	17	14	2.5	2.5	2.0	—

The dimensions of the coalesced trees which we investigated are given in the table.

The distance between the coalesced trees was 2-5 m. These "coalescence hybrids" exhibit clearly defined morphological and anatomical differences from the controls. A description of these differences has already been given in previously published papers [8,9]. The course of transpiration throughout a 24 hour period is given in the graphs (figure, A). The curves show the transpiration levels for both controls and common pine/Crimean pine "coalescence hybrids".

It is obvious from the graphs that the Crimean pine (control) exercises greater economy in its transpiration loss than does the common pine (control). In the former tree, the transpiration gradually increases in proportion to the increased air temperature and the reduction in relative humidity. The highest level of transpiration in this species occurs during the hottest period of the day. By contrast, the common pine exhibits its most intensive transpiration during the morning and consequently, by the time the day is at its hottest, its water reserves are severely depleted and the level of transpiration falls sharply.

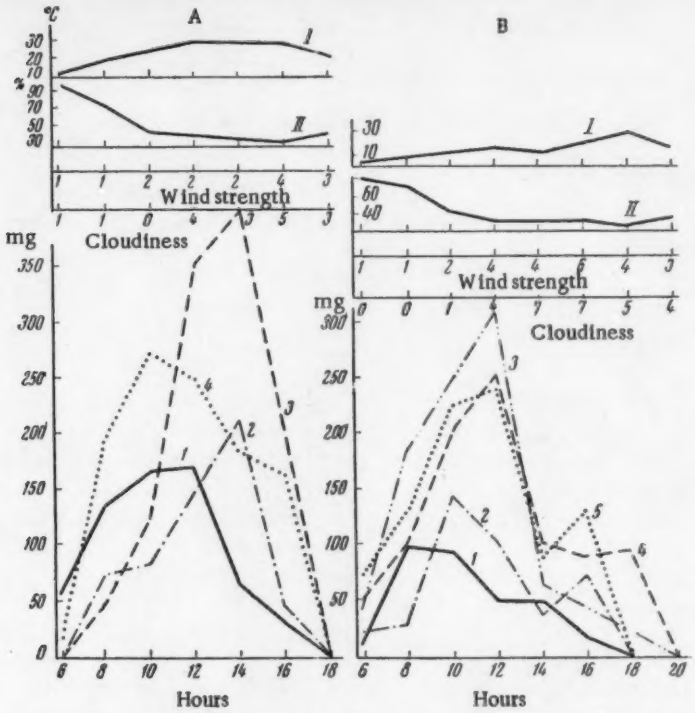
In the case of the common pine on Crimean pine "hybrid," the level of transpiration is approximately 2.5 times that of the control (common pine), but the form of the diurnal curve approximates more closely to that of the Crimean pine.

In the case of the Crimean pine on common pine "hybrid," the level of transpiration is again somewhat higher than that of the controls. Its transpiration curve is a fusion, as it were, of the curves of the two different controls. It would appear that the pattern of transpiration represented by this curve is particularly favorable for the tree in question, for its transpiration rate remains high throughout the day and its nutritional level, as indicated by strong growth, is correspondingly high (see table).

Examples of coalescence between Bank's pine and common pine were observed in a mixed 35-year-old plantation. The plantation consisted of 50% Bank's pine and 50% common pine trees. Density of planting was 0.7-0.8. Investigations into diurnal rhythm of transpiration were carried out on example No. 1, which consisted of three trees manifesting coalescence of their growth. Two of these trees were common pines; the third was a Bank's pine.

The data given in the lower half of the table indicates that the "coalescence hybrid" trees were larger than the controls and grow more rapidly. Moreover, as in the previously discussed case, the "coalescence hybrid" exhibits clearly defined anatomical and morphological features.

The diurnal sequence of transpiration is shown in the graph (figure, B). The curve for this sequence of transpiration in the Bank's pine (control) possesses two peaks. In general, the rate of transpiration for this species is higher than for the common pine. The curve for the Bank's pine on common pine "hybrid" also possesses two peaks, but the intensity of transpiration is approximately twice that of the controls. The common pine on Bank's pine "hybrids" again show a considerably higher level of transpiration than the controls. In one of these individuals (situated nearer to Bank's pine trunk



Transpiration of "coalescence hybrids" and control trees throughout the day. A) The Crimean pine/common pine "hybrid": 1) common pine (control); 2) Crimean pine (control); 3) common pine growing on Crimean pine; 4) Crimean pine growing on common pine. B) The Bank's pine/common pine "hybrid": 1) common pine (control); 2) Bank's pine (control); 3) common pine No. 1 growing on Bank's pine; 4) common pine No. 2 growing on Bank's pine; 5) Bank's pine growing on common pine. I) Temperature of air; II) relative humidity of air.

than the other), the diurnal transpiration curve, although having two peaks, has the second peak much more feebly developed than the first.

The high intensity of transpiration manifested by these "coalescence hybrids" testifies to their ability to withstand arid conditions and to their considerable vigor and rapid growth.

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*MdDDG – Mitteilungen der Deutschen Dendrologischen Gesellschaft.

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THE CHEMICAL STRUCTURE AND HERBICIDAL ACTIVITY OF SOME DERIVATIVES OF PHENYLHYDROXYLAMINE

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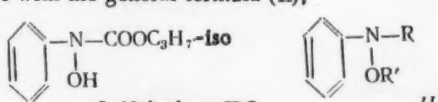
The physiological activity of hydroxylamine derivatives has not been studied until recently. In 1943 Chabrolin [1], seeking new herbicides, showed herbicidal activity in hydroxylamine sulfate and other compounds of various structures whose water solutions at a concentration of 5% destroyed annual plants: field radish, corn poppy, striped melon, and others. However, in this case the herbicidal action was evidently due to the acid component of the salt.

Aside from this, hydroxylamine plays a large role in the vital activities of plants, taking part in the assimilation of nitrates and nitrites and the synthesis of amino acids [2]. In this connection it would be expected that hydroxylamine derivatives would also intervene in analogous metabolic processes, showing various effects on the growth and development of plants.

In studying the herbicidal action of hydroxy derivatives of isopropyl phenylcarbamate, the author, along with Mel'nikov and Zemskaya [3, 4], showed the high activity of N-hydroxyisopropylphenylcarbamate (N-hydroxyIPC) (I), which can be considered as an acyl derivative of phenylhydroxylamine. In this connection we studied for the first time the physiological activity of a large group of acyl derivatives of phenylhydroxylamine with the general formula (II),



I, N-hydroxyIPC

II

in which R is the residue of a physiologically active or inactive acid (benzoic, phenylacetic, α-naphthylacetic, 2,4-dichlorophenoxyacetic, or 2,4,5-trichlorophenoxyacetic) and R' can be R or hydrogen.

Compounds and Method for Studying their Physiological Activity

All the compounds except N-benzoylhydroxylamine were undescribed in the literature and were obtained by the action of the corresponding acid halide on phenylhydroxylamine in a water solution in the presence of an acid binding material. For study we used the analytically pure form of the substances.

The study of the physiological activity of the compounds was carried out on sprouting wheat seeds of the variety Lutescence 62, and on radishes of the Rose-Red variety, according to the method described earlier [5]. The substances were studied in the form of 1 and 5% dusts with a norm of utilization of 25 mg of dust per 50 grains. The study was repeated twice. We measured the length of the leaves and maximum length of the root in the wheat sprouts, and the length of the stem and root stalk for the radish sprouts.

The degree of inhibition was calculated with respect to the controls and was expressed conditionally according to the following scale:

Growth stimulation 66-33% (– –)
Absence of inhibition or weak growth stimulation 33-0% (–)
Weak inhibiting action 0-33% (+)
Inhibiting action 33-66% (+ +)
Strong growth inhibition or death of plant 66-100% (+ + +)

The results of the study are given in Tables 1 and 2.

As the data in Table 1 show, all of the studied derivatives of phenylhydroxylamine which contain physiologically inactive or slightly active herbicidal groups have no herbicidal activity or have it to a very slight extent. This applies to all the compounds which contain as substituents residues of benzoic, phenylacetic, and α-naphthylacetic acids. Of these compounds, only N-benzoyl-O-α-naphthylacetylphenylhydroxylamine, which contains the α-naphthylacetic acid moiety on the hydroxyl group, shows a selective herbicidal action on the radish at the maximum concentration studied.

Results and Discussion

The introduction into the phenylhydroxylamine molecule of a residue of 2,4-dichlorophenoxyacetic or 2,4,5-trichlorophenoxyacetic acid gives a substance with a high degree of physiological activity.

Compounds which contain residues of 2,4-dichlorophenoxyacetic or 2,4,5-trichlorophenoxyacetic acid on the nitrogen show a sharply selective action on dicotyledenous plants. The selectivity in the action of this

TABLE 1. Herbicidal Activity of Phenylhydroxylamine Derivatives

No.	Phenylhydroxylamine	M. p., °C	Dust concentration, %	Degree of growth inhibition			
				wheat		radish	
				stem	root	stem	root
1	N-Benzoyl	121—122	5	—	—	+	++
2	N-Phenylacetyl	89—92	1	—	—	+	+
			5	—	+	+	++
3	N-Benzoyl-O-phenylacetyl	68—70	1	—	+	—	+
			5	—	+	—	+
4	N-Phenylacetyl-O-benzoyl	131—132	1	—	+	—	+
			5	+	++	+	+
5	N,O-Bis(phenylacetyl)	76—78	1	——	—	—	—
			5	——	+	+	+
6	N-α-Naphthylacetyl	120.5—122	1	—	—	+	+
			5	—	+	+	++
7	N-Benzoyl-O-α-naphthylacetyl	93—95	1	—	+	+	+
			5	—	++	+++	+++
8	N-2,4-Dichlorophenoxyacetyl	167—168	1	+	++	+++	+++
			5	++	+++	+++	+++
9	N-Benzoyl-O-2,4-dichlorophenoxyacetyl	87—88	1	+++	+++	+++	+++
			5	+++	+++	+++	+++
10	N-2,4-Dichlorophenoxyacetyl-O-benzoyl	119—122	1	—	+	++	+++
			5	+	+	+++	+++
11	N-Phenylacetyl-O-2,4-dichlorophenoxyacetyl	106—107	1	+	+++	+++	+++
			5	++	+++	+++	+++
12	N-2,4-Dichlorophenoxyacetyl-O-phenylacetyl	108—109.5	1	—	+	+++	+++
			5	+	++	+++	+++
13	N,O-Bis(2,4-dichlorophenoxyacetyl)	113—115	1	++	+++	+++	+++
			5	++	+++	+++	+++
14	N-2,4,5-Trichlorophenoxyacetyl	183—184	1	—	+	+++	+++
			5	—	++	+++	+++
15	N-Benzoyl-O-2,4,5-trichlorophenoxyacetyl	103—105	1	+++	+++	+++	+++
			5	+++	+++	+++	+++
16	N-2,4,5-Trichlorophenoxyacetyl-O-benzoyl	113—114	1	—	+	++	++
			5	+	+	++	++
17	N-2,4,5-trichlorophenoxyacetyl-O-phenylacetyl	119—120	1	—	+	++	+++
			5	—	+	+++	+++
18	N-2,4-Dichlorophenoxyacetyl-O-2,4,5-trichlorophenoxyacetyl	112—114	1	+	++	+++	+++
			5	++	+++	+++	+++

group of substances rises considerably without decrease in effectiveness of herbicidal action on radish sprouts when the hydrogen of the hydroxyl group in the molecule is replaced by the benzoyl or phenylacetyl groups.

Phenylhydroxylamine derivatives in which the halophenoxyacetyl group replaces the hydrogen of the hydroxyl group surpass the corresponding N-halophenoxyacetyl derivatives in activity as herbicides, but yield to them in selective action. As the data of Table 2 show, these compounds are close in physiological activity to

TABLE 2. Comparative Physiological Activity of Halophenoxy Derivatives of Phenylhydroxylamine and the Corresponding Halophenoxyacetic Acids

Compound	Dust concentration, 10^{-3} mole %	Degree of growth inhibition			
		wheat		radish	
		stem	root	stem	root
N-Phenylacetyl-0-2,4-dichlorophenoxyacetylphenylhydroxylamine	0.45	+	+ +	+ + +	+ + +
2,4-Dichlorophenoxyacetic acid	0.45	+ +	+ + +	+ + +	+ + +
N-Benzoyl-0-2,4,5-trichlorophenoxyacetylphenylhydroxylamine	0.39	–	+ +	+ + +	+ + +
2,4,5-Trichlorophenoxyacetic acid	0.39	+ + +	+ + +	+ + +	+ + +

the corresponding halophenoxyacetic acids used in equimolar amounts, and in some cases surpass them in selective action.

The difference in degree of toxicity in the selective action of 0- and N-halophenoxyacetylphenylhydroxylamines is obviously explained by the difference in stability in the plants: compounds in which the 2,4-dichlorophenoxyacetyl or 2,4,5-trichlorophenoxyacetyl residue is bound through an ester link are considerably more labile than compounds with an amide bond.

To clarify the prospects for use of the halophenoxyacetylphenylhydroxylamines as herbicides of a selective or general action, it will be important to carry out further testing of the compounds which show high activity in the preliminary tests. In particular it is of interest to study the preparations by the method of previous introduction into the soil, that is, under conditions in which 2,4-dichlorophenoxyacetic acid is comparatively ineffective.

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METHODS

APPARATUS FOR STUDYING TRANSIENT PHENOMENA OF PHOTOSYNTHESIS BY MEANS OF THE CARBON RADIOISOTOPE C^{14}

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On sudden illumination of photosynthetic material the conjointly acting biophysical and chemical processes responsible for the assimilation of radiant light energy in photosynthesis are for a certain period of time in a state of development, during which the different links "team up," until there sets in a steady coordinated unbalance of all the systems, which directs the process as a whole along a strictly determined course. This state of the photosynthetic process is called the "induction period of photosynthesis."

Later, when the process is in the steady state, the phenomena typical of the induction period of photosynthesis are concealed by the over-all course of rapid carbon dioxide reduction. At this time the "end effects" – CO_2 absorption, oxygen evolution, accumulation of carbon reduction products –are clearly observed, but the intermediate mechanisms responsible for this process are concealed. Hence, a study of the transient phenomena of photosynthesis, which are observed in the first minute after illumination of the object or when it is darkened, are of great importance for an understanding of the internal mechanism of photosynthesis. The information amassed in this way will be, as Rabinowitch concludes, "a fundamental contribution toward the general edifice of photosynthesis – and biochemistry in general – as an exact science" [1].

It has been established in investigations of induction phenomena that in the first minute after the onset of illumination there is a rapid absorption of carbon dioxide (the so-called "gulp" of CO_2) and at this time, or close to it, there is a "gush" of oxygen, a sudden conversion of inorganic phosphorus to organic, and a diminution of fluorescence and absorption at 515 mμ.

The CO_2 absorption curve then descends, and similar curves illustrate the kinetics of O_2 evolution, phosphorus conversion, fluorescence, chemiluminescence, and so on [1-3]. Thus, the basic course of the processes has been established. However, it is still difficult to correlate the many individual observations, much less to explain them in terms of any simple mechanism. This is largely attributable to the fact that the methods used for studying induction effects often do not allow an interpretation of the obtained results. In particular, this is what we find in an examination of the data relating to the kinetics of CO_2 absorption in the induction period of photosynthesis, since such measurements are made in apparatuses in which a current of air passes through the chamber containing the object and the analysis of the CO_2 content is made on a differential basis [4-7]. Hence, for instance, it is impossible to say what is the cause of the specific drop in the CO_2 absorption curve after the initial "gulp." Is there a cessation of CO_2

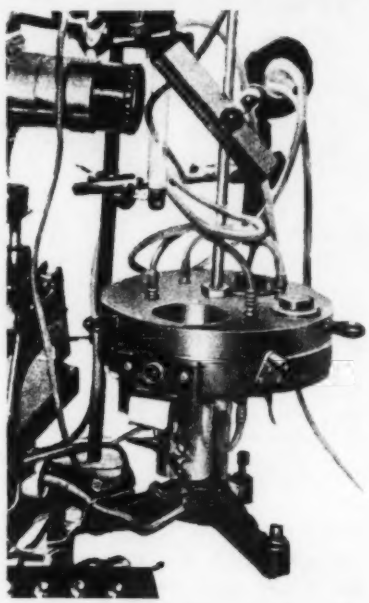

Fig. 1. Apparatus for study of transient phenomena of photosynthesis by means of carbon radioisotope C^{14}.

absorption (for instance, owing to the exhaustion of the CO_2 acceptor stored up in the dark) some time after the onset of illumination, or in this case is there a reverse evolution of CO_2 due, say, to incorporation in the chain of oxidative reactions. A solution of this question alone is important, especially now, when Warburg has advanced his hypothesis of energy dismutation in the process of photosynthesis [8], and discussions of the quantum yields of photosynthesis and its chemistry have been taken up again with renewed vigor [1, 9, 10].

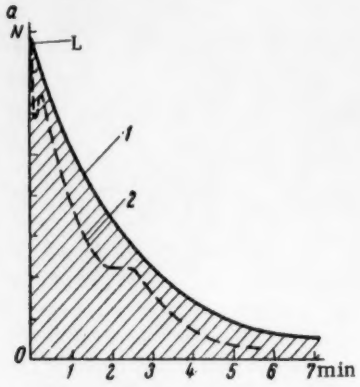

Fig. 2. Fall of radioactivity in closed chamber on exposure of green leaf in it. 1) Registered course of fall of radioactivity in chamber; 2) region inaccessible to measurement; possible actual course of kinetics of C^{14} absorption in induction period of photosynthesis. L) Light.

Using the carbon radioisotope C^{14} we made an attempt to investigate some of the unclarified questions which might correlate the established effects or which might allow an unambiguous interpretation of them so that we could discover what mechanisms are concealed behind the specific behavior of the curves of the induction period of photosynthesis.

By means of an apparatus specially designed for this purpose (Fig. 1) we succeeded in showing [3] that during the induction period of photosynthesis, the rapid absorption of carbon dioxide was followed by a gush of some of the CO_2 absorbed in the previous moment, and not by a cessation of its absorption, as was the accepted view. We studied the transient phenomena of photosynthesis which are observed on transition from light to darkness.

One of the main parts of the apparatus is the special chamber for exposing the object to $C^{14}O_2$.

In a study of the question from the viewpoint of method we settled on the use of a closed chamber for exposing the object, because in this case all the registered changes in CO_2 content (as distinct from differential gas analyzers with a flow of gas) would be due solely to the activity of the green leaf contained in the chamber. By the employment of the carbon radioisotope C^{14} as an indicator all the changes in CO_2 content could be continuously measured and registered on a recorder. Closed chambers for exposing the object with the use of C^{14} have been used several times for a study of photosynthesis [11-13], and have been shown to be

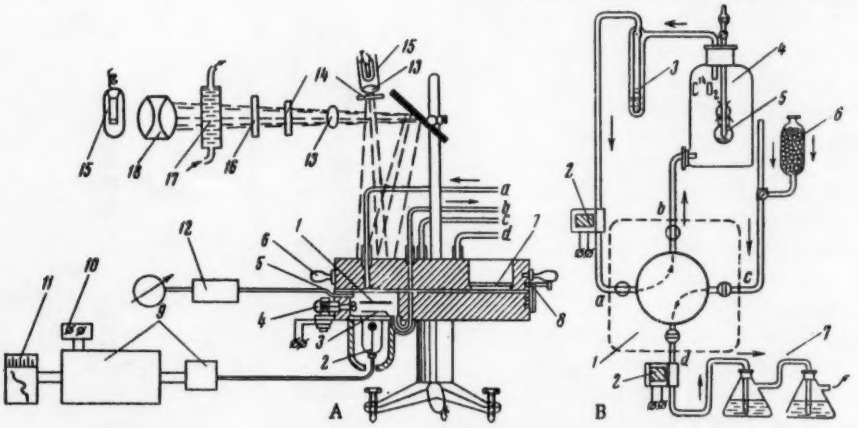

Fig. 3. Diagram of apparatus. A) Chamber for exposure: 1) Leaf; 2) end-window counter; 3) screen; 4) fan; 5) thermistor; 6) handle; 7) glass window; 8) lock; 9) scaler; 10) mechanical pulse counter; 11) recorder; 12) temperature control unit; 13) objectives; 14) neutral filters; 15) lamps; 16) color filter; 17) heat filter; 18) condenser. B) Gas flow system: 1) Exposure chamber; 2) diaphragm pumps; 3) rheometer; 4) bottle with gaseous $C^{14}O_2$; 5) ampoule with radioactive sodium carbonate; 6) carbon dioxide absorber; 7) $C^{14}O_2$ absorber; a, b, c, d) branch tubes.

fully suitable for this purpose. However, as calculations made for the chambers described in the literature have shown, the ratios of the active photosynthesizing leaf surface to the volume of gas in the chambers to the concentration and specific activity of carbon dioxide in them are such that the slight changes in radioactivity which are observed in the first instant after the onset of illumination are concealed in a region inaccessible to measurements owing to the general high background of radioactivity in the chamber at the onset of exposure (Fig. 2). In order that these changes in C^{14} content (if they exist) at the onset of illumination of the leaf can be detected, the size of the chamber has to be such that the ratio of the quantity of carbon dioxide absorbed in 1 min by a given area of leaf, at a given intensity of photosynthesis, to the quantity of CO_2 in the chamber is 0.1-1. The design of the chamber and the whole apparatus was based on this initial requirement. The assembled apparatus consists of a special chamber for exposing the object, illumination systems, a system for filling the chamber with $C^{14}O_2$ and absorption of the used activity, and an electronics unit with an automatic recorder to provide a continuous trace of the activity changes in the chamber (Fig. 3).

The chamber for exposing the object consists of two metal (steel and fine cast iron) disks, 190 mm in diam and 25 mm thick, which are ground to fit one another so that when the upper disk is rotated relative to the lower their planes of contact still provide an air-tight seal. The lower disk is rigidly mounted on a stand. The upper disk can rotate around its axis. The leaf chamber proper is in the lower disk and consists of an eccentrically bored hole into the bottom of which is screwed the rim of an end-window Geiger-Müller counter so that the rim of the counter and its window form the actual bottom of the chamber.

The depth of the chamber is 13 mm, its diameter 55 mm, and volume 29.44 cm^3. The thickness of the

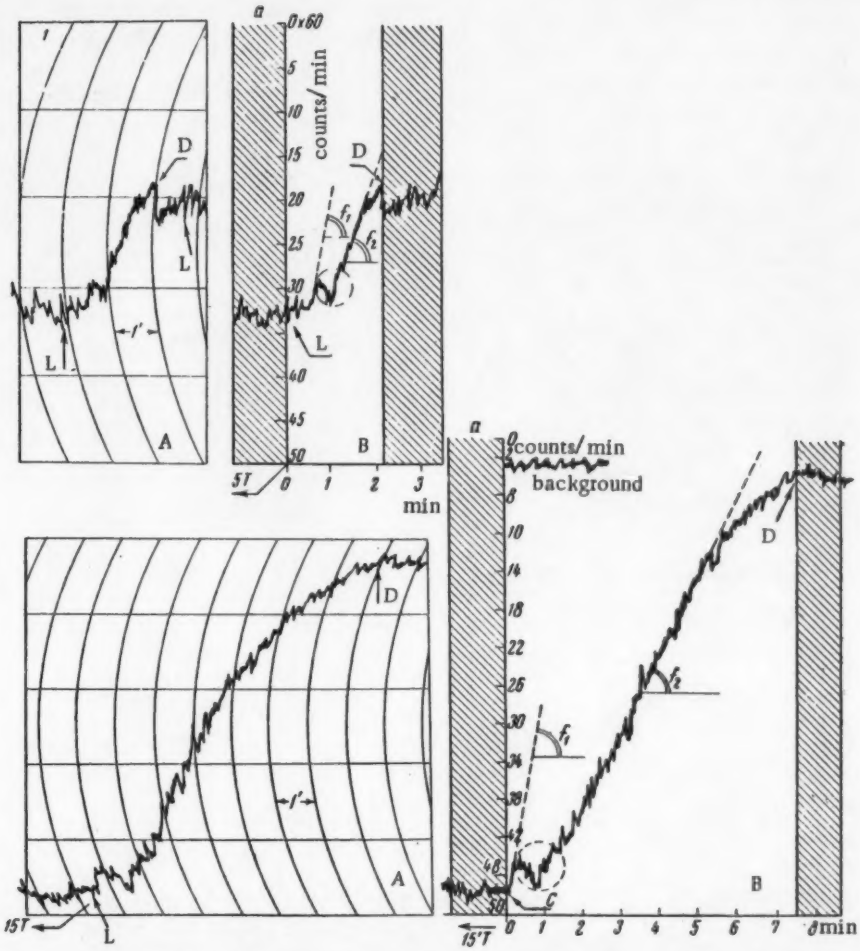

Fig. 4. Kinetics of $C^{14}O_2$ absorption in induction period of photosynthesis in Helianthus annuus. A) Trace diagrams; B) curves converted to rectangular coordinate system. L) light; D) dark.

mica window of the end-window counter used is 2 mg/cm^2, and the diameter of the window 25 mm. Fastened inside the chamber above the counter window there is a brass foil disk screening the counter from particles of C^{14} already fixed by the leaf. The effective counting volume between the counter window and the screen is 2.45 cm^3. Thus, the end-window counter inserted in the chamber could register only the radioactivity of the gas in the chamber. A 19.53 cm^2 portion of leaf was mounted in a special holder above the screen.

To avoid stationary pockets of gas in the chamber and to ensure a uniform distribution of $C^{14}O_2$ throughout the whole chamber, a miniature electromagnetic fan similar to that used by Zalenskii,et al. [12] is inserted in the chamber. In addition, the chamber also contains a needle thermistor for temperature control.

In the bottom of the chamber there is an opening which communicates through a connection piece and tube with a duct leading to the plane of contact of the two disks. In the upper disk there are two pairs of ducts with branch tubes, arranged so that when the upper disk is in certain positions, set by means of a lock, the ducts coincide with the leaf chamber and the duct in the lower disk (as shown in Fig. 3). In these positions of the upper disk the chamber can either be filled with $C^{14}O_2$ by pumping in a specially prepared mixture of gas containing $C^{14}O_2$, or the chamber can be flushed and the $C^{14}O_2$ absorbed by alkali. In addition, the upper disk has an opening for the insertion of the leaf in the chamber and a glass window. Thus, all the operations with the chamber—insertion of leaf, filling with $C^{14}O_2$, illumination, darkening, $C^{14}O_2$ absorption—can be carried out by rotating and setting the upper disk in fixed positions.

The branch tubes a and b (Fig. 3) of the upper disk are connected to a closed cycle in which a current of gas for filling the chamber with $C^{14}O_2$ is created by means of an airtight diaphragm electromagnetic pump. $C^{14}O_2$ of known specific activity is generated in a bottle (capacity 2 liters) included in the system. The other two branch tubes (c and d) are connected with another diaphragm pump and a $C^{14}O_2$ absorption system. The illuminating systems are similar to those which we used earlier [7].

The changes of radioactivity in the chamber are registered and recorded by means of a scaler with an automatic recording instrument on the H 370-A 1500 μa output. The lag time of the whole system is 25-35 sec.

A piece of leaf is placed in the chamber, which is filled with gas containing a known content of $C^{12}O_2$ and $C^{14}O_2$ and, depending on the object of the investigation, is illuminated, darkened or subjected to other effects.

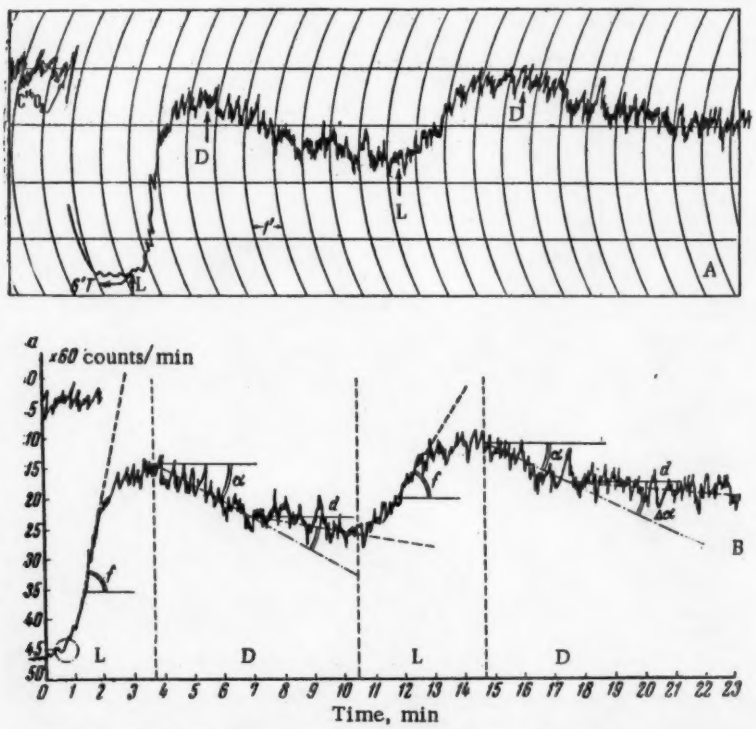

Fig. 5. Transient phenomena of photosynthesis in Helianthus annuus on transition from light to dark. A) Trace diagram; B) curve converted to rectangular coordinate system; L) light; D) dark.

Examples of traces of the kinetics of $C^{14}O_2$ absorption in the induction period of photosynthesis and of the transient phenomena of photosynthesis which are observed on transition from light to dark are given in Figs. 4 and 5, which show the original trace diagrams and the treatment of the obtained curves. The recording instrument used gave a trace in a distorted coordinate system. Hence, for a quantitative analysis of the obtained data the traces were transferred to a rectangular coordinate system by means of specially constructed plotters and rulers.

In the case of our chamber, with the given parameters induction and transition phenomena are observed with CO_2 concentrations of 0.035 to 1% in the chamber, specific activity 5 to 0.7 $\mu C/mg\ CO_2$, and light intensities of 20 to $50 \cdot 10^3$ lux, respectively. In this case the rate of photosynthesis of the leaf had to be about 25-30 $mg/dm^2/hr$ or more. Such an object was Helianthus, distinguished by a high rate of photosynthesis, which becomes saturated with light at light intensities of around 400×10^3 $erg/cm^2/sec$ [14].

I express my deep thanks to Prof. A. A. Nichiporovich for numerous discussions on the problem and his guidance of this work.

SUMMARY

An apparatus for studying the mechanism of transition processes in photosynthesis induced by sudden illumination or darkening of green leaves is described.

The apparatus in essence consists of an air-tight leaf chamber which contains an end-window counter, small fan and temperature sensitive element. The chamber can be illuminated. It is connected to a system of diaphragm pumps and shutters which permit rapid changes of the gas in the chamber. The chamber volume, leaf area and CO_2 concentration are chosen in such a way that it is possible to study the induction effects by measuring the C^{14} activity. Variations in the radioactivity in the chamber during photosynthesis are recorded automatically.

By aid of the apparatus it has been demonstrated that after a rapid CO_2 "gulp" observed directly after the beginning of illumination, part of the CO_2 absorbed during the "gulp" is evolved, the rate of this "gush" being about 60% of the absorption rate. Transition phenomena accompanying the change from light to darkness were investigated.

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METHOD OF STUDYING RATE OF WATER EXCHANGE (TOTAL COMSUMPTION OF WATER BY SOIL AND PLANT) IN FIELD AND LABORATORY CONDITIONS

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Translated from Fiziologiya Rastenii, Vol. 8, No. 1,
pp. 134-137, January-February, 1961
Original article submitted February 14, 1959

In the works of Genkel'[1-3] the view was developed that the ecology of water exchange in plants was dissimilar in different groups of xerophytes. In this connection the problem of drought resistance of plants can be resolved if field methods of studying the rate of water exchange in different groups of plants can be perfected [4]. Proceeding from this, Sabinin [5] expressed the conviction that in the future the most valuable methods for solving the problem of drought resistance would be those methods of studying xerophytes which would enable the obtention of a more or less reliable curve for the daily consumption of water by plants in conditions of different types of drought. He wrote: "It is necessary to investigate the curve of the transpiration rate and rate of consumption of water reserves against a background of varying values of saturation deficiency, solar radiation intensity, and wind speed. So long as we lack measurements of this kind the solution of several questions of the physiology of the water relations of xerophytes will be of an extremely approximate nature" (p. 163).

We tested the following type of instrument (figure), which was constructed by P. P. Teterin. In devising the technique and in constructing the instrument we took into consideration the results of our investigations on the special features of the water exchange in Stipa-xerophytes [5-8], hemixerophytes [9], and euxerophytes [10], and also the possibility of using in the field a method which we suggested earlier [11]. For the investigation of succulents and poikiloxerophytes we will apparently have to introduce additional improvements to the apparatus described below.

To determine the rate of water exchange (water consumption by soil and plant) we use an essentially physical method, which permits a reasonably precise measurement of the rate of consumption of water by soil and plants in field conditions. The essence of the method consists in the following: Suppose that in the reservoir A (see figure) containing water there is a drop in water level. The shift in water level will cause a movement of the float C connected to the end of lever d. The other end of lever d rotates freely around a horizontal axle O. Valve m rests on the lever. The shift of float C causes the opening or closing of the orifice for admitting water into reservoir A. When water from the valve chamber K passes into reservoir A, the float C is raised and the valve orifice admitting the water is closed. With such an arrangement a constant water level can be maintained in reservoir A. The quantity of water passing into reservoir A from reservoir Q can be determined with sufficient accuracy from the difference in the readings of the position of the tip of the pointer on the kymograph tape from the formula $q = K(n_1 - n)$, where q is the quantity of water flowing out of reservoir Q into reservoir A; K is a proportionality factor; n_1 and n are the initial and final positions of the tip of the pointer on the kymograph tape.

The apparatus consists of the following parts (see figure): A cylinder D of diam 9 cm and height 16 cm, which provides the water for the initial filling of the pores and capillaries of the investigated soil samples, communicates through a rubber tube with vessel B. Vessel B is a truncated cone with diameters 17 and 10 cm and height 18 cm. It is constructed of fired clay with a coating of oil paint and contains the test samples of soil. Vessel B is connected by a tube through cock Z to the brass cylinder A, which is 8 cm in diam and 10 cm high. Soldered to the side wall of cylinder A, as the sketch shows, is the valve chamber K (outer diam 1 cm, inner 0.8 cm, height 5 cm), which is connected by a socket σ to the float chamber Q, which has an internal diam of 7 cm and a height of 9 cm. The valve m (diam 0.2 cm) is well ground to fit the seat of the valve chamber and rests freely on the lever d (the lever has an arm ratio of 1:5). One end of lever d rotates around the horizontal axle O fastened to the valve chamber, while the other is rigidly connected to float C. The vertical rod of float ρ, contained in the float chamber Q, bears against the center of the base of cylinder Z_1. Cylinder Z_1 is rigidly attached to the pointer, which has an arm ratio 1:7. The pointer p rotates freely between centers around the horizontal axis O'. The rotation of the pointer

is regulated by a weight P so that the slight friction of the tip of the pointer against the lightly smoked ruled tape of the kymograph N is overcome. The time is plotted on the horizontal lines of the kymograph tape and the mass of evaporated water in grams is plotted on the vertical arcs.

After all the parts of the apparatus are connected up the whole system is filled with water with cocks 1 and Z closed. The level of water in cylinders A and D is set 0.5 cm above the bottom of vessel B, with valve m preventing admission of water into reservoir A. The float chamber is filled with water until the tip of the pointer coincides with the upper horizontal line of the kymograph tape. When all the parts of the apparatus are filled with water cock 1 is opened, the water rises into the capillaries, and the humidity of the surface of the soil sample varies for three to four hours and then remains constant.

We measured the humidity of the soil surface with adequate accuracy by our own method [10] and the "Universal moisture meter.

When the soil moisture becomes constant, the cock 1 is closed and simultaneously cock Z is opened. The tip of the pointer is then brought against the moving tape of the kymograph and the curve of water consumption in relation to time is obtained. From the obtained curve the mean or true rate of water consumption by the soil and plants can be determined.

Sensitivity of Apparatus

By the sensitivity of the apparatus we mean its ability to detect the mass of water in grams evaporated from 1 cm^2 of soil surface and we denote it by $n = \frac{m}{S}$ where n is the sensitivity, m the mass of water in grams, and S the surface of the body in cm^2.

With an evaporating surface of 226 cm^2 our apparatus had a sensitivity of $n = 0.001$ g/cm^2.

Before the vessels are packed with soil their inside surface is coated with a film of molten paraffin, and the outside of the vessels is enameled.

No. of expt.	Quantity of water	Position of pointer tip	
		initial	final
1	25	0	50
2	20	0	40.2
3	15	0	30.4
4	10	0	20.2
5	5	0	10.0
6	13	0	26.3
7	18	0	36.2
8	22	0	44.0
9	7	0	14.1
10	3	0	6.0

Calibration of Apparatus

The apparatus is calibrated in the following manner. After setting the tip of the pointer on the uppermost horizontal line (called the initial reading—n) of the kymograph tape, we remove 25 g of water from reservoir A and read off the position, denoted by n_1, of the tip of the pointer on the kymograph tape. By adding water to the float chamber we then return the tip of the pointer to the initial reading and remove another 20 g of water and read off the position of the end of the pointer on the tape. Such experiments were conducted numerous times with different quantities of water. As an illustration we give the results of one series of experiments in the table.

An examination of the data shown in the table shows that in a first approximation the difference of readings ($n_1 - n$) of the position of the pointer tip on the kymograph tape is directly dependent on the quantity of water removed from reservoir A. For instance, a difference in readings $n_1 - n = 50$ scale divisions corresponds to 25 g of water, a difference in readings $n_1 - n = 10$ scale divisions corresponds to 5 g of water, and so on.

If we assume now that the number of divisions through which the pointer shifts in a vertical direction is known, the quantity of water which has passed out of the float chamber can be found with adequate accuracy

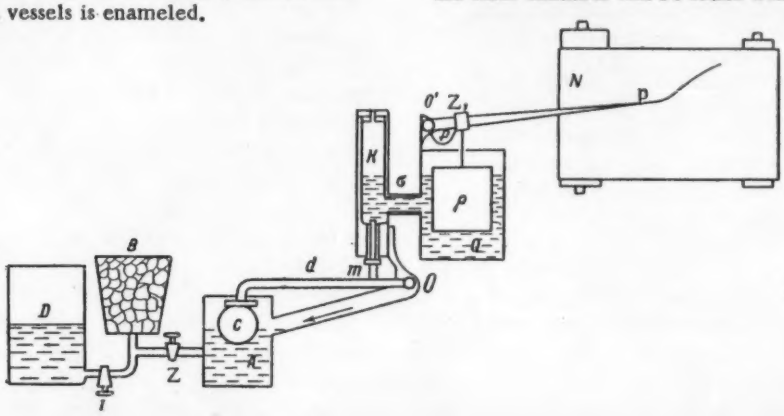

Diagram of instrument for investigating rate of water exchange in field and laboratory conditions. (Explanation in text.)

from the formula $q = K_1 (n_1 - n)$, where K_1 is the proportionality factor for our apparatus, $K_1 = \frac{1}{2}$ g. Having determined the mass of the water entering the soil of the test sample and the period of time of entry of the water from the kymograph tape, we can easily find the rate of water consumption from the formula

$$Q = \frac{K_1(n_1 - n)}{t}$$

where Q is the mass of the water consumed per hr and t is the time in hours.

It follows from the foregoing account that the method we have devised for determining the rate of water consumption allows a sufficiently accurate determination of the water consumption by soil and plants in relation to environmental conditions and time.

The results of our investigations on the rates of water consumption by soil and plants (we do not give the kymograms of the rate of water consumption by soil and plants) are easily differentiated by the methods we have devised. Among the number of advantages of the proposed method we should count the relative simplicity of construction and the possibility of using it both in field and laboratory conditions. Several vessels with experimental plants can be connected at one time to the above-described apparatus. The vessels for the experiments can be prepared in various ways: water culture, sand culture, filling the vessel with sod with intact soil structure and with growing live plants of a particular ecological group, and so on. The presence of an automatic recorder in the instrument rules out the need for periodic weighing of the vessels, since the apparatus at any time of the day gives the water consumption by the soil and plant in absolute units and at the same time gives an objective curve of the daily variation of the rate of water exchange at constant humidity. In view of all these features we can recommend the above-described method as better than existing potometers and other indirect methods of estimating water exchange [4, 6, 7, 12-15, et al.].

The results of the application of our method to the study of Stipa-xerophytes and other ecological groups of plants will be communicated by us in papers dealing with a characterization of the various groups of plants of the arid steppes of Stavropol.

SUMMARY

A new type of apparatus is described for studying water metabolism in plants and the rate of evaporation of water from the soil.

The instrument can be employed to measure the amount of water directly absorbed in vessels with the experimental plants. The vessels for the experiments may be prepared in a variety of ways. Thus, one may use soil cultures, water cultures or natural sod with intact soil structure and living plants.

The main details of the apparatus are shown in the figure. The amount of water expended by the plant and soil is measured with the help of floating chamber A and valve m. A float with a rod connected by levers with the driving axis of recorder O' is placed within the floating chamber.

Because of the recorder there is no need to weigh the vessels periodically, as the instrument at any time yields in absolute units the expenditure of water due to evaporation by the plant and soil.

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REFRACTOMETRIC METHOD OF ESTIMATING WATER CONTENT OF LEAVES OF APPLE TREES

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The refractometric method has found wide application in the study of the water relations of plants [1-4]. In view of the several advantages of this method for field studies we investigated the feasibility of using it for assessing the water relations of apple trees. For this purpose we made numerous determinations of the concentration of the cell sap in leaves in comparison with their water content.

The concentration of the leaf cell sap was determined by means of the field refractometer Mark RP Kiev. A drop of juice extruded by means of a special hand press from one or two leaves wrapped up in a piece of gauze was deposited on the prism of the refractometer and the index of refraction was read off. The cell sap concentration was expressed in arbitrary units expressing its optical activity in terms of the optical activity of sucrose solutions of a particular concentration. At the same time we determined the air temperature so that the appropriate corrections to the refractometer readings could be made. The water content of the leaves was determined by drying them to constant weight in a drying cabinet at 100-105°. For comparative determinations we selected equally illuminated leaves on the same or similarly situated fruiting branches in the crown of the tree. The measurements were made in four replicates.

The leaf samples were taken from apple trees of different varieties on different stocks and at ages 2, 10, and 20 years—seedlings on vigorous stocks in the second field of the nursery; young trees on vigorous stocks, just starting to fruit; 20-year-old trees on dwarf stocks (Paradise IX), with and without fruit. We chose leaves subjected to different water conditions due to the hydrological conditions in which the trees were growing and to the age of the trees, the position of the leaves in the crown of the tree and their degree of illumination, and also to the times of measurement during the day and the growing period. Thus, for comparison we tried to include the cell sap concentration and water content of leaves under the greatest variety of water conditions. The investigations were carried out in 1958 and 1959 in the Moldavian Research Institute of Horticulture, Viticulture and Wine-Making.

The results of comparisons of the cell sap concentrations and water content of spur shoot leaves of several varieties of apple trees from measurements made in 1959 are shown in the figure.

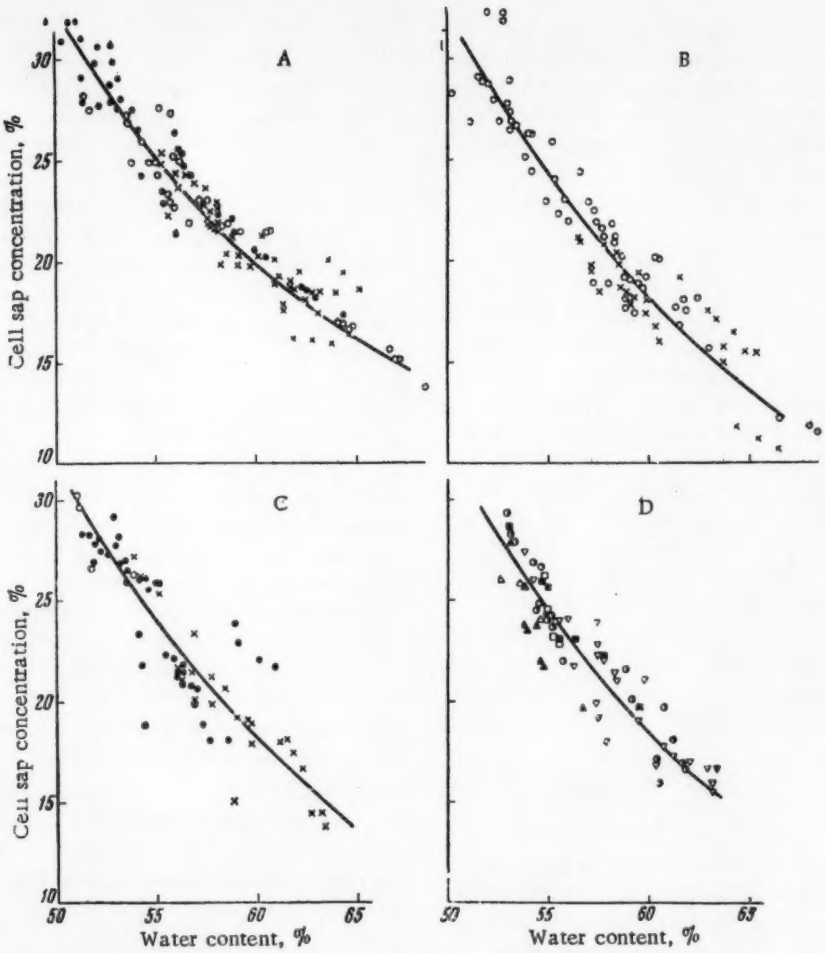
In all the varieties which we studied we found a very close relationship between these indices. An increase in cell sap concentration was accompanied by a reduction in water content. The curves of this relationship consisted of hyperbolas with equally large radii of curvature for all the varieties. The scatter of the experimentally determined points around the curves for all varieties was slight in the absolute majority of cases. Individual large deviations were probably accidental and were due either to errors of a technical nature or to insufficient care in choosing spur shoot leaves in regard to their conditions of illumination throughout the day. The variety of objects, conditions and times of sampling the leaves for the determinations had no significant effects on this relationship. A very important fact was that the scatter in the figure containing the data for six varieties of apple trees with pronounced differences in biological features and times of ripening of the fruit was the same as in the individually taken varieties Wagner's Prize, White Winter Colville and Champagne Reinette.

The quantitative relationship between the cell sap concentration and the water content of the leaves was the same in almost all the varieties. A cell sap concentration of 20% corresponded to a water content of about 58.5% of the dry weight, and a concentration of 30-31% corresponded to 50-51%. Only in the variety Wagner's Prize was this relationship modified, particularly in the lower part of the curve. In this variety a cell sap concentration of 20% corresponded to a leaf water content of about 60%.

The coefficients of correlation between the cell sap concentrations and the water content in the spur shoot leaves confirmed the existence of a very reliable re-

lationship between these indices for all varieties, as the following data for 1959 show:

Variety	Number of compared measurements (n)	Coefficient of correlation (r ± mr)
Wagner's Prize	120	-0.93±0.013
White Winter Colville	83	-0.94±0.013
Champagne Reinette	67	-0.89±0.025
Golden Winter Pearmain Simirenko Reinette, Kandil' Sinap, Summer Saffron, Landsberg's Reinette, Papirovka	68	-0.91±0.022



Relation between cell sap concentration and water content in spur shoot leaves of various varieties of apple trees. A) Wagner's Prize; B) White Winter Colville; C) Champagne Reinette; D) Group of varieties. Symbols for curves A, B, C: dark circles—trees without fruit; light circles—trees with fruit; crosses—seedlings and young trees, just starting to fruit. Symbols for curve D for group of varieties: triangle with black dot inside—Golden Winter Pearmain; circle with cross inside—Simirenko Reinette; black triangle—Landsberg's Reinette; white triangle—Summer Saffron; white square—Kandil' Sinap; square with cross inside—Papirovka.

Similar results for the tested varieties Wagner's Prize and White Winter Colville were obtained in 1958 as well. The negative coefficients of correlation between these indices were 0.90-0.93 with the same high degree of reliability.

Thus, the cell sap concentration, determined by the refractometer, of leaves of spur shoots of apple trees is a reliable physiological index of their water content.

The values of the cell sap concentration of these leaves from the quantitative aspect were comparable with one another in the estimation of the state of their water content. This comparability was due to the similar age of the leaves of a spur shoot, the growth of which in apple trees terminates early in spring, soon after the buds open.

In view of the almost identical quantitative relationship between the cell sap concentration and the water content of leaves and the same nature of the curves of this relationship in the investigated varieties, we can regard the cell sap concentration of leaves as a fairly universal index of their water content for all or at least most of the varieties of apple trees. As regards an estimation of the water content of leaves of growth shoots from their cell sap concentration, this is rather complicated by their different age along the length of the shoot. However, the coefficients of correlation between the cell sap concentration and the water content in leaves of growth shoots of the varieties investigated were also high and were 0.80-0.85 with a high degree of accuracy.

Our investigations showed that the refractometer values of the cell sap concentration of leaves of apple trees agree quantitatively with the dry matter content of the juice determined by drying to constant weight. In accordance with the well known equation giving the osmotic pressure of solutions in relation to their concentration, the concentration of the cell sap of apple leaves fairly reliably characterizes its osmotic pressure.

Since there is an inverse relationship between the water content of leaves and their water deficiency, we can postulate just as close a relationship between the cell sap concentrations of apple leaves and their water deficiency. All this indicates the feasibility of using the refractometric method for estimating the state of the water relations of individual leaves of apple trees, or of different parts of the crown, or of the tree as a whole. A fault of this method is that in several cases there is not always a distinct line of separation in the refractometer field, especially at high cell sap concentrations. However, with some familiarity in conducting the readings this fault of the method can easily be overcome. The laboratory refractometer Mark RL-2 (Kiev) gives more definite readings, although it is less convenient and not so easily operated for measurements in field conditions. A very distinct line of separation in determinations of the concentration of cell sap from freshly plucked leaves is provided by the Polish field refractometer Mark RR-1, of improved design. The use of this refractometer provides reliable readings and every convenience in field work.

SUMMARY

The possibility of employing a field refractometer for estimating the water content of apple tree leaves was investigated. A high negative correlation has been established between the cell sap concentration and water content in leaves of the nine apple varieties studied. The cell sap concentration determined by aid of the refractometer yields reliable information on the water content of the leaves. The refractometric method is a simple and convenient means for studying the water content in the apple tree under field conditions.

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CURRENT EVENTS

THE FIRST SIBERIAN CONFERENCE OF PLANT PHYSIOLOGISTS AND BIOCHEMISTS

L. D. Prusakov

Translated from Fiziologiya Rastenii, Vol. 8, No. 1,
pp. 141-142, January-February, 1961

The first conference of physiologists and biochemists of Siberia was called September 6-9, 1960, by an initiative group of Irkutskian physiologists and biochemists of the Eastern Siberian branch of the Siberian division of the Academy of Sciences, USSR. Over one hundred specialists came to Irkutsk from Moscow, Leningrad, Siberia, Sakhalin and the Far East. The issues of the conference came from a complex of scientific problems which scientists of Siberia and the Far East had to solve. Professor F. E. Reimers, director of the biology division of the Eastern Siberian branch of the Academy of Sciences, USSR, and a doctor of biological sciences, on opening the conference spoke on the problems and prospects of the development of plant physiology and biochemistry of Siberia arising from the rapid rate of the utilization of her natural resources.

The chief problems are:

a) in the area of physiological problems—the solution of problems of the resistance of plants to unfavorable environmental conditions, beginning with frost and winter resistance for vast Siberian forest and subforest zones, drought resistance for steppe districts, and the study of the processes of plant growth and development under the specific conditions of Siberia. It is absolutely certain that in order to solve these leading problems, it is necessary to work out the problems of air and soil nutrition under local conditions;

b) the problems of the biochemistry of the wood species constituting the vast Siberian forest, the study of substances of high physiological activity, and the study of local plant resources are probably the most important theoretical and practical problems of biochemical investigations in Siberia.

This theme of biological investigations also determined the program of the conference.

In the plenary sessions of the conference 11 general reports devoted to basic problems of plant physiology in Siberia (nutrition, photosynthesis, plant growth and development, resistance, and water relations) were heard.

Many reports gave rise to lively discussion: D. I. Sapozhnikov's "The participation of carotenoids in photosynthetic reactions"; B. S. Moshkov's "Cultivation of plants under artificial illumination"; the paper by M. M. Okuntsov, et al., "The effect of separate parts of the spectrum on plant respiration"; the paper by N. S. Petinov, L. D. Prusakovaya and K. M. Malyshevaya, "Some problems of the physiology of irrigated plants under conditions in Kulanda"; and Z. I. Zhurbitskii's "Physiological and agrochemical bases for the use of fertilizers."

In addition to the plenary sessions, the work of the conference was carried out in two sections: 1) atmospheric and mineral nutrition and biochemistry, where 25 reports were heard and discussed; and 2) growth, development and resistance, with 27 reports.

It can be noted with satisfaction that in Irkutsk there is a growing tendency to study auxin changes in plants (V. V. Polevoi). Reports by O. A. Zauralov and R. M. Tarkhanov were devoted to the study of cold resistance in plants—the solution of a problem so important for Siberia; a report by I. S. Buddo was devoted to the study of water relations in plants under conditions in Irkutsk. Works carried out on mineral nutrition under Siberian conditions were reflected in reports by N. K. Boldyrev, G. V. Barinov, N. M. Maiboroda, and M. V. Efimov.

All reports were the result of original experimental work, and we can state with satisfaction that there has been a growth, on an experimental basis, in a number of new scientific centers of Altai, the Far East, and Buryatia.

The scope of the problems discussed in the sections naturally was very broad, beginning with photosynthesis and mineral nutrition and ending with problems of the biochemistry of nectar. The most attention was allotted to the study of growth under different conditions in Siberia and to the role of artificial illumination in the cultivation of plants in Siberia. Together with the working out of a number of theoretical problems (A. V. Krylov, "Plant growth and magnetism"), attention was drawn to the practical end toward which the majority of the works were directed. We would like to note the

most thorough investigations in the use of the newest methods: N. G. Doman, T. F. Andreevaya. Of the works having a practical importance, we should name those of K. P. Bolginaya, L. P. Romanovaya, L. N. Bereznegovskaya, L. S. Alyukinaya, V. G. Minaevaya, and A. I. Yakubovaya.

As a result of thediscussions conducted on the various problems of plant physiology and biochemistry developing in Siberia, a resolution was adopted, the text of which is published below.

Resolution of the First Siberian Conference of Plant Physiologists and Biochemists, held on September 6-9, 1960, in the City of Irkutsk

The present stage of thedevelopment of communism in the USSR is characterized by exceptionally fast rates of utilization of the vast natural resources of Siberia, agricultural and forest resources among them. The development of these sections of the national economy, in a very large number of cases fully inevitable, requires a broad development of investigations in plant physiology and biochemistry, which forms a theoretical basis for the successful cultivation of agricultural crops and for the exploitation of the forest and other plant wealth of Siberia.

In recent years a considerable increase has been noted in the number of scientific institutions in which projects are being carried out in plant physiology and biochemistry. The cadre of physiologists and biochemists in Siberia has grown considerably.

The creation of a Siberian division of the Academy of Sciences has a very great importance for the development of biological science, particularly plant physiology and biochemistry, in Siberia.

The frequency of contacts between individual scientists and scientific organizations has grown, which improves the prospects for coordinating investigations, and,in particular, led to the calling of this conference.

Together with this, the conference noted that investigations in physiology and biochemistry are still insufficiently developed in Siberia, and not to the extent where they correspond to the needs of agriculture and forestry.

As yet there have not been enough continual contacts or coordination of investigative plans achieved between scientific institutions, particularly between research projects of institutions of the Academy of Sciences, USSR, and universities, on the one hand, and the network of experimental agricultural institutions on the other hand.

Having heard and discussed in the plenary and sectional sessions reports of the learned physiologists and biochemists of Siberia and of the central research institutions of the Academy of Sciences, USSR, of the Lenin All-Union Agricultural Academy, of universities, and of the network of scientific stations for agriculture and forestry, the conference resolves:

1. To recognize the working out of the following basic problems of physiology and biochemistry in the different ecogeographical regions of Siberia as being undertakings of primary importance:

a) for the western steppe and forest steppe zone of Siberia—problems of mineral nutrition (in connection with soil microbiology), of salt resistance, of the water relations in plants, and of irrigation;

b) for the zone of the Middle Siberian Plateau—problems of photosynthesis, of cold and winter resistance of plants, of plant growth and development, and of the physiology of the forest species constituting the vast Siberian forest;

c) for the Transbaikal—problems of drought resistance, irrigation, and mineral nutrition, especially in the area of the study of microelements;

d) for the Far East—problems of the physiology of growth and development, mineral nutrition, the physiology of the diseased plant, and the physiology of forest species;

e) for the Arctic North of Siberia—problems of plant growth and development in permafrost, and problems of photosynthesis under local conditions.

It is natural that, in addition to these trends determined by the ecogeographical environment, in each zone the general questions of plant physiology necessary for the solution of concrete practical problems, or arising in the process of investigations, must also be developed.

The conference attaches particular importance to working out the problems of the culture of plants under artificial light for the arctic zones of Siberia, to the study of the physiology of plants cultivated in greenhouses and hotbeds for Central Siberia, and also to the creation of detailed ecophysiological characteristics of the basic agricultural crops in a plan of regulations for shaping the harvest of their productive sections. It would be highly desirable for projects of the latter nature to be included in the research programs of all agricultural research institutions in Siberia (zonal institutes and regional agricultural experimental stations).

2. It is absolutely necessary to enlarge the number of institutions in which research work in plant physiology and biochemistry is being carried out. In particular, it is very desirable,in the next year or two, to set up within the system of the Siberian division of the Academy of Sciences, USSR, an Institute of Plant Physiology and Biochemistry which would be the natural coordinating center of biological sciences in this region.

The conference places with the Ministry of Agriculture and the All-Union Academy of Agricultural Sciences an urgent request for the creation of divisions and laboratories of plant physiology and biochemistry in all agricultural research institutes and in experimental and plant-breeding stations.

3. It is necessary to accelerate considerably the training of cadres of physiologists and biochemists through graduate work, both in the Siberian research institutions and also in the institutes of Moscow and Leningrad which enter into the system of the Academy of Sciences, USSR, and the All-Union Academy of Agricultural Sciences.

Those participating in the conference appeal to the scientists of Moscow and Leningrad with a request for the rendering of assistance in training cadres of plant physiologists and biochemists for the research institutions in Siberia.

4. For the successful development of investigations in plant physiology and biochemistry on the present scientific level, it is necessary to build and equip artificial climate laboratories as soon as possible in at least three points in Siberia: Western (the city of Novosibirsk), Eastern (the city of Irkutsk), and in the Far East (the city of Vladivostok or Khabarovsk).

5. It is extremely vital to set up an experimental factory in Siberia for the production of laboratory equipment, and to set up trade in reagents and apparatus in the larger cities.

6. For the fulfillment of the resolution of the botanical conference held in 1957, to request the Siberian division of the Academy of Sciences, USSR, to intercede before the Presidium of the Academy of Sciences, USSR regarding the publication of a periodic biological journal in Siberia.

7. To request the Siberian division to publish the proceedings of the present conference.

RECOLLECTIONS OF ARKADII IVANOVICH POTAPOV

P. A. Genkel'

Translated from Fiziologiya Rastenii, Vol. 8, No. 1,
pp. 143-144, January-February, 1961

On May 21, 1960, one of the last of V. I. Palladin's students and a doctor of biological sciences, Professor A. I. Potapov, died suddenly.

A. I. Potapov was born January 28, 1882, in the village of Malaya Bykovka of Pugachev region in Kuibyshev district. His father was the village schoolmaster After finishing secondary school in the city of Samara, Arkadii Ivanovich entered Yur'ev University in 1904, and in 1907 proceeded to the Faculty of Physics and Mathematics at Peterburg University, where he graduated in 1912. At the University A. I. Potapov specialized in the department of plant physiology under Professor V. I. Palladin in problems of plant respiration. As a result of this work A. I. Potapov published the article, "The effect of products of autolysis on the respiratory quotients of plants" [1]. After graduating, Arkadii Ivanovich worked in Novouzensk (Samar district) and later Ufimsk district on national education. Only after the Great October Socialistic Revolution in 1920 did he find his true vocation and become a scientific worker.

In 1920 A. I. Potapov took the position of assistant at Irkutsk University. A year later he moved to the Tulunsk Experimental Station, where he worked successfully until 1924, and then became a lecturer at Irkutsk University.

At the Tulunsk Experimental Station, Arkadii Ivanovich worked productively on problems of physiology, agricultural chemistry, and plant protection. He worked on the culture of the tannin-producing plant Bergenia crassifolia (1928, 1929); he developed a biological method of combating thistle by infecting it with rust fungi (1925); he studied the acidity of Siberian forest soils (1928); and he worked out a combination method for nitrogen fixation (1928). In addition to this, A. I. Potapov published a series of manuals dealing with actual problems of farming in Siberia (the fungicide treatment of potatoes, iodine deficiency in water and forage, new models for the reclaiming of marsh lands, and others).

Of his investigations carried out in Siberia, we should note an article on the physiological features of wheat xerophytes (1924). In this work Arkadii Ivanovich demonstrated experimentally the untenableness of the opinion of V. R. Zalenskii on the loss of wheat from dampness and dryness under natural conditions as a result of an irreversible opening of the stomatal apparatus.

In 1929 I. E. Znamenskii, in a laboratory supervised by A. I. Potapov, established the high toxicity of aluminum sulfate for wheat. Developing this trend further during his activity at Gruziya, Arkadii Ivanovich defined the toxic role of aluminum ions for the tea bush in red soils, and suggested the use of large doses of phosphate fertilizers as one of the measures for combating soil acidity. We should note that the works of A. I. Potapov on the study of the tea plant were not only concerned with the study of soil acidity, but dealt with a variety of problems: the effect of growth stimulators on growth acceleration, the role of tyrosinase in the technology of tea, a new method of storing tea seeds, the study of the root system of the tea bush, the role of the drying process in tea technology, weedy vegetation of tea plantations, and other problems.

During the last years of his life A. I. Potapov continued his investigations in connection with the toxic role of the aluminum already in the podzolic soils of Podmoskov with a number of his students (P. D. Bukharin, N. Elizarova, Yu. Petrenko). A. I. Potapov presented a summary of this material in an article published in 1956, "Increasing the fertility of acid soils by a method of chemical melioration."

In 1930, A. I. Potapov was admitted to the academic rank of professor, and, in 1934, to the academic degree of doctor of biological sciences.

After his departure from Irkutsk University (1929) A. I. Potapov worked in a number of cities of our country (Saratov, Vladikavkaz, Ozurgety, Kuibyshev, Pskov, and Moscow).

From 1934 to 1938, A. I. Potapov worked in the position of senior scientific collaborator in the K. A. Timiryazev Plant Physiology Institute, Academy of Sciences, USSR, and from 1943 to 1947 in the Laboratory for Evolutionary Ecology, Academy of Sciences, USSR.

From 1947 to 1954, A. I. Potapov was a professor at the Moscow District Educational Institute.

After 1954, A. I. Potapov went on pension and engaged in literature work. Having been in education, and through the nature of his activity in plant physiology, A. I. Potapov, working many years at experimental institutions, gave much of his attention to agricultural chemistry and also to problems of plant protection. All this enabled him, during the last years of his life, to give successfully the course "Fundamentals of Agri-

culture" at the Moscow District Educational Institute. The textbook "Fundamentals of Agriculture" was prepared by A. I. Potapov in collaboration with V. Mosolov (Uchpedgiz, 1949). In addition, "Let Us Practice Agriculture" was written by him. This was a textbook for students of educational institutes (Uchpedgiz, 1952,1958).

A. I. Potapov conducted the teaching of the course on the fundamentals of agriculture on a broad biological basis, taking into consideration the specific nature of this subject in educational institutions.

A. I. Potapov was a man who sharply felt the need for a close connection between scientific theory and practice. Possibly this also explains the diversity of the subjects of his scientific works. All his scientific works were carried out in response to the demands of practice, and had the most direct connection with it. In all, about 50 scientific works and other articles were published by him.

All who knew A. I. Potapov will always remember him best as a good and sincere man and comrade.

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ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci-Tech Press
GOST	All Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LEM	Laboratory for experimental morphogenesis
LENDVI	Leningrad Inst. of Dermatology and Venereology
LEO	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci. Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIEM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.

Soviet Journals Available in Cover-to-Cover Translation

ABBREVIATION	RUSSIAN TITLE	TITLE OF TRANSLATION	PUBLISHER	TRANSLATION BEGAN Vol.	Issue	Year
AÉ	Atomnaya énergiya	Soviet Journal of Atomic Energy	Consultants Bureau	1	1	1956
Akust. zh.	Akusticheskii zhurnal	Soviet Physics – Acoustics	American Institute of Physics	1	1	1955
	Antibiotiki	Antibiotics	Consultants Bureau	4	1	1959
Astr(on). zh(urn).	Astronomicheskii zhurnal	Soviet Astronomy–AJ	American Institute of Physics	34	1	1957
Avto(mat). svarka	Avtomaticheskaya svarka	Automatic Welding	British Welding Research Association (London)		1	1959
	Avtomatika i Telemekhanika	Automation and Remote Control	Instrument Society of America	27	1	1956
	Biofizika	Biophysics	National Institutes of Health*		1	1957
	Biokhimiya	Biochemistry	Consultants Bureau	21	1	1956
Byull. éksp(erim). biol. i med.	Byulleten' éksperimental'noi biologii i meditsiny	Bulletin of Experimental Biology and Medicine	Consultants Bureau	41	1	1959
DAN (SSSR) } Dokl(ady) AN SSSR }	Doklady Akademii Nauk SSSR	The translation of this journal is published in sections, as follows:				
		Doklady Biochemistry Section	American Institute of Biological Sciences	106	1	1956
		Doklady Biological Sciences Sections (Includes: Anatomy, biophysics, cytology, ecology, embryology, endocrinology, evolutionary morphology, genetics, histology, hydrobiology microbiology, morphology, parasitology, physiology, zoology sections)	American Institute of Biological Sciences	112	1	1957
		Doklady Botanical Sciences Sections (Includes: Botany, phytopathology, plant anatomy, plant ecology, plant embryology, plant physiology, plant morphology sections)	American Institute of Biological Sciences	112	1	1957
		Proceedings of the Academy of Sciences of the USSR, Section: Chemical Technology	Consultants Bureau	106	1	1956
		Proceedings of the Academy of Sciences of the USSR, Section: Chemistry	Consultants Bureau	106	1	1956
		Proceedings of the Academy of Sciences of the USSR, Section: Physical Chemistry	Consultants Bureau	112	1	1957
		Doklady Earth Sciences Sections (Includes: Geochemistry, geology, geophysics, hydrogeology, mineralogy, paleontology, petrography, permafrost sections)	American Geological Institute	124	1	1959
		Proceedings of the Academy of Sciences of the USSR, Section: Geochemistry	Consultants Bureau	106-123	1 6	1957- 1958
		Proceedings of the Academy of Sciences of the USSR, Sections: Geology	Consultants Bureau	106-123	1 6	1957- 1958
		Doklady Soviet Mathematics	The American Mathematics Society	131	1	1961
		Soviet Physics–Doklady (Includes: Aerodynamics, astronomy, crystallography, cybernetics and control theory, electrical engineering, energetics, fluid mechanics, heat engineering, hydraulics, mathematical physics, mechanics, physics, technical physics, theory of elasticity sections)	American Institute of Physics	106	1	1956
		Proceedings of the Academy of Sciences of the USSR, Applied Physics Sections (does not include mathematical physics or physics sections)	Consultants Bureau	106-117	1	1956-1957
Derevoobrabat. prom-st'.	Derevoobrabatyvayushchaya promyshlennost'	Wood Processing Industry	Timber Development Association (London)		9	1959
	Élecktrosvyaz	Telecommunications	Massachusetts Institute of Technology*		1	1957
Éntom(ol). oboz(renie)	Éntomologicheskoe obozrenie	Entomological Review	American Institute of Biological Sciences	38	1	1959
Farmakol. (i) toksikol(ogiya)	Farmakologiya i toksikologiya	Pharmacology and Toxicology	Consultants Bureau	20	1	1957
FMM	Fizika metallov i metallovedenie	Physics of Metals and Metallography	Acta Metallurgica*	5	1	1957
Fiziol. zhurn. SSSR (im. Sechenova)	Fiziologicheskii zhurnal im. I. M. Sechenova	Sechenov Physiological Journal USSR	National Institutes of Health*		1	1957
Fiziol(ogiya) rast.	Fiziologiya rastenii	Plant Physiology	American Institute of Biological Sciences	4	1	1957
	Geokhimiya	Geochemistry	The Geochemical Society		1	1958
FTT	Fizika tverdogo tela	Soviet Physics–Solid State	American Institute of Physics	1	1	1959
Izmerit. tekh(nika)	Izmeritel'naya tekhnika	Measurement Techniques	Instrument Society of America		1	1959
Izv. AN SSSR, O(td). Kh(im). N(auk)	Izvestiya Akademii Nauk SSSR: Otdelenie khimicheskikh nauk	Bulletin of the Academy of Sciences of the USSR: Division of Chemical Sciences	Consultants Bureau		1	1952

continued

Izv. AN SSSR, O(td). T(ekhn). N(auk): Met(all). i top.	(see Met. i top.)					
Izv. AN SSSR Ser. fiz(ich).	Izvestiya Akademii Nauk SSSR: Seriya fizicheskaya	Bulletin of the Academy of Sciences of the USSR: Physical Series	Columbia Technical Translations		1	1954
Izv. AN SSSR Ser. geofiz.	Izvestiya Akademii Nauk SSSR: Seriya geofizicheskaya	Bulletin (Izvestiya) of the Academy of Sciences USSR: Geophysics Series	American Geophysical Union		1	1954
Izv. AN SSSR Ser. geol.	Izvestiya Akademii Nauk SSSR: Seriya geologicheskaya	Izvestiya of the Academy of Sciences of the USSR: Geologic Series	American Geological Institute		1	1958
Kauch. i rez.	Kauchuk i rezina	Soviet Rubber Technology	Research Association of British Rubber Manufacturers	18	1	1959
	Kinetika i kataliz	Kinetics and Catalysis	Consultants Bureau	1	3	1960
	Koks i khimiya	Coke and Chemistry USSR	Coal Tar Research Association (Leeds, England)		1	1958
Kolloidn. zh(urn).	Kolloidnyi zhurnal	Colloid Journal	Consultants Bureau	14	1	1952
	Kristallografiya	Soviet Physics – Crystallography	American Institute of Physics	2	1	1957
Metalov. i term. obrabot. metal.	Metallovedenie i termicheskaya obrabotka metallov	Metal Science and Heat Treatment of Metals	Acta Metallurgica	6	1	1958
	Metallurg	Metallurgist	Acta Metallurgica		1	1957
Met. i top.	Metallurgiya i topliva	Russian Metallurgy and Fuels	Eagle Technical Publications		1	1960
Mikrobiol.	Mikrobiologiya	Microbiology	American Institute of Biological Sciences	26	1	1957
OS	Optika i spektroskopiya	Optics and Spectroscopy	American Institute of Physics		6	1959
	Pochvovedenie	Soviet Soil Science	American Institute of Biological Sciences		1	1958
	Priborostroenie	Instrument Construction	British Scientific Instrument Research Association		1	1959
Pribory i tekhn. éks(perimenta)	Pribory i tekhnika éksperimenta	Instruments and Experimental Techniques	Instrument Society of America		1	1957
Prikl. matem. i mekh.	Prikladnaya matematika i mekhanika	Applied Mathematics and Mechanics	American Society of Mechanical Engineers		1	1958
PTÉ	(see Pribory i tekhn. éks.)					
	Problemy Severa	Problems of the North	National Research Council of Canada			
Radiotekh.	Radiotekhnika	Radio Engineering	Massachusetts Institute of Technology*	12	1	1957
Radiotekh. i élektronika	Radiotekhnika i élektronika	Radio Engineering and Electronics	Massachusetts Institute of Technology*	2	1	1957
	Stanki i instrument	Machines and Tooling	Production Engineering Research Assoc.		1	1959
	Stal'	Stal (In English)	Iron and Steel Institute		1	1959
Stek. i keram.	Steklo i keramika	Glass and Ceramics	Consultants Bureau	13	1	1956
Svaroch. proiz-vo	Svarochnoe proizvodstvo	Welding Production	British Welding Research Association		4	1959
Teor. veroyat. i prim.	Teoriya veroyatnostei i ee primenenie	Theory of Probability and Its Applications	Society for Industrial and Applied Mathematics		1	1956
Tsvet. Metally	Tsvetnye metally	Nonferrous Metals	Primary Sources		1	1960
UFN	Uspekhi fizicheskikh Nauk	Soviet Physics – Uspekhi (partial translation)	American Institute of Physics	66	1	1958
UKh	Uspekhi khimii	Russian Chemical Reviews	The Chemical Society (London)		1	1960
UMN	Uspekhi matematicheskikh nauk	Russian Mathematical Surveys	London Mathematical Society	15	1	1960
Usp. fiz. nauk	(see UFN)					
Usp. khim(ii)	(see UKh)					
Usp. matem. nauk	(see UMN)					
Usp. sovr. biol.	Uspekhi sovremennoi biologii	Russian Review of Biology	Oliver and Boyd		48	1959
Vest. mashinostroeniya	Vestnik mashinostroeniya	Russian Engineering Journal	Production Engineering Research Assoc.		4	1959
Vop. gem. i per. krovi	Voprosy gematologii i perelivaniya krovi	Problems of Hematology and Blood Transfusion	National Institutes of Health*		1	1957
Vop. onk.	Voprosy onkologii	Problems of Oncology	National Institutes of Health*		1	1957
Vop. virusol.	Voprosy virusologii	Problems of Virology	National Institutes of Health*		1	1957
Zav(odsk). lab(oratoriya)	Zavodskaya laboratoriya	Industrial Laboratory	Instrument Society of America	25	1	1959
ZhAKh Zh. anal(it). khimii	Zhurnal analiticheskoi khimii	Journal of Analytical Chemistry USSR	Consultants Bureau	7	1	1952
ZhETF Zh. éksperim. i teor. fiz.	Zhurnal éksperimental'noi i theoreticheskoi fiziki	Soviet Physics–JETP	American Institute of Physics	28	1	1955
ZhFKh Zh. fiz. khimii	Zhurnal fizicheskoi khimii	Russian Journal of Physical Chemistry	The Chemical Society (London)		7	1959
ZhMÉI Zh(urn). mikrobiol. épidemiol. i immunobiol.	Zhurnal mikrobiologii, épidemiologii i immunobiologii	Journal of Microbiology, Epidemiology and Immunobiology	National Institutes of Health*		1	1957
ZhNKh Zh(urn). neorgan(ich). khim(ii)	Zhurnal neorganicheskoi khimii	The Russian Journal of Inorganic Chemistry	The Chemical Society (London)		1	1959
ZhOKh Zh(urn). obshch(ei) khimii	Zhurnal obshchei khimii	Journal of General Chemistry USSR	Consultants Bureau	19	1	1949
ZhPKh Zh(urn). prikl. khimii	Zhurnal prikladnoi khimii	Journal of Applied Chemistry USSR	Consultants Bureau	23	1	1950
ZhSKh Zh(urn). strukt. khimii	Zhurnal strukturnoi khimii	Journal of Structural Chemistry	Consultants Bureau	1	1	1960
ZhTF Zh(urn). tekhn. fiz.	Zhurnal teknicheskoi fiziki	Soviet Physics–Technical Physics	American Institute of Physics	26	1	1956
Zh(urn). vyssh. nervn. deyat. (im. Pavlova)	Zhurnal vysshei nervnoi deyatel'nosti (im. I. P. Pavlova)	Pavlov Journal of Higher Nervous Activity	National Institutes of Health*		1	1958

*Sponsoring organization. Translation through 1960 issues is a publication of Pergamon Press.

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